PROCEEDINGS

OF THE

INDIAN ACADEMY OF SCIENCES

VOL. XXII

SECTION B

BANGALORE CITY
PRINTED AT THE BANGALORE PRESS, MYSORE ROAD
1946

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ON A NEW SPECIES OF GENUS *OOCHORISTICA* FROM THE INTESTINE OF *CALOTES VERSICOLOR*

By V. R. Misra, M.Sc.

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Received January 10, 1945

[Communicated by Dr. G. S. Thapar, M.Sc., Ph.D. (London), F.A.Sc.]

Oochoristica indica SP. NOVO.

About 50 species have been described under the genus *Oochoristica* from reptilian and mammalian hosts, the reptilian host being more common. The chief differences among various species are:—size of the worms, size of scolex and suckers, presence or absence of receptaculum seminis, size, extent and course of cirrus sac, number of testes and their arrangement, character and shape of vitelline glands, and the size and arrangement of eggs in gravid segment.

The worms described here have been obtained from the small intestine of *Calotes versicolor* in Lucknow. Out of four animals dissected only one was infected. They were killed by hot water, fixed in formalin and preserved in 3% formalin to which a drop of glycerine was added.

The worms were stained with Acetic Alum Carmine and Borax Carmine but the latter did not give satisfactory results.

External characters.—The length of the worms ranges between 136 and 200 mm. with a maximum breadth of 1·35 to 1·55 mm. Scolex is about 350 μ in diameter and the suckers, 170 to 190 μ, are cup-shaped and muscular, of which two are placed on the dorsal side and two on the ventral. A neck measuring 6 mm. long is followed by segmentating strobila. In immature segments, rudiments of cirrus sac and vagina appear first and about the same time rudiments of ovary also appear. After about 30 immature segments, mature ones are encountered. The immature segments are broader than long but these become longer as we proceed backwards in the strobila.

The genital pores are irregularly alternate. In 61 fully mature consecutive segments the arrangement of genital pores was found thus:—6 R, 2 L, 3 R, 3 L, 1 R, 6 L, 1 R, 2 L, 3 R, 4 L, 1 R, 1 L, 4 R, 6 L, 1 R, 3 L, 2 R, 1 L, 3 R, 1 L, 1 R, 1 L, 1 R, 1 L, 2 R, 1 L = 61 segments.

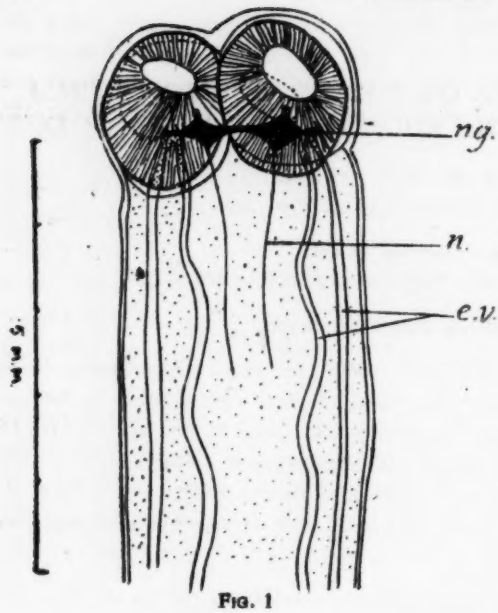

FIG. 1

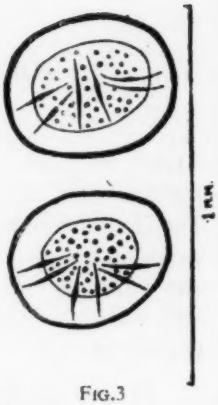

FIG. 3

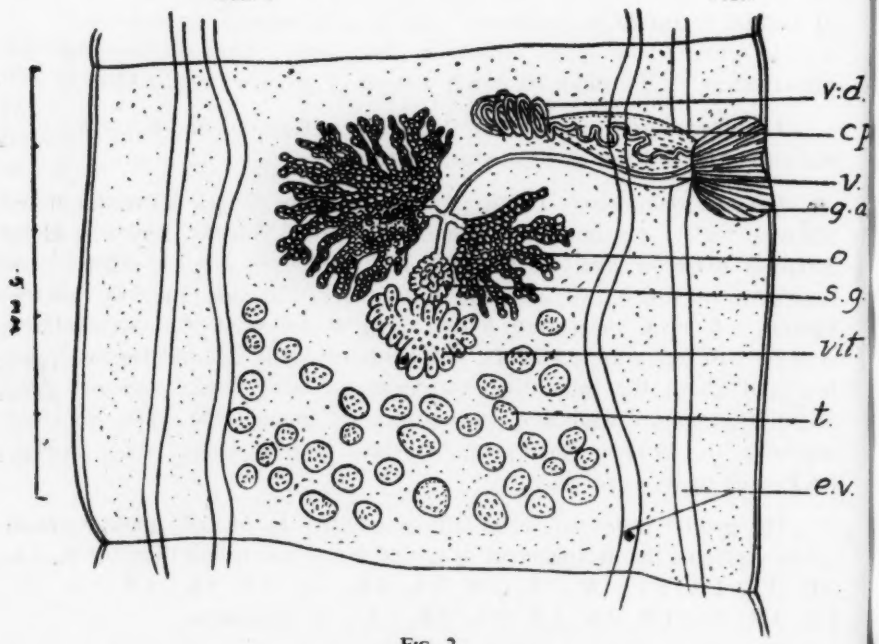

FIG. 2

FIGS. 1-3.—Fig. 1. Scolex. Fig. 2. A mature segment. Fig. 3. Two eggs showing membranes and hooks.

c.p., Cirrus pouch; *e.v.*, Excretory vessels; *g.a.*, Genital atrium; *n.*, Nerve ; *n.g.*, Nerve ganglion; *o.*, Ovary; *s.g.*, Shell gland; *t.*, Testes; *v.*, Vagina; *v.d.*, Vas deferens; *vit.*, Vitelline gland.

Excretory system.—There are four excretory canals, two being on either side. The outer ones are nearly straight tubes but the inner ones are wavy. The two vessels of either side unite in the scolex just behind the suckers after which they ramify. The vessels run as two separate ducts and they do not inter-communicate nor have transverse connections at the posterior end of the segment.

Nervous system.—Two nerve ganglia are present between the dorsal and ventral suckers. They give out two nerve cords, one from each ganglion, on the posterior side. The nerve cords run parallel to the inner excretory duct.

Reproductive system.—Reproductive organs are simple. There is a muscular oval genital atrium in which open the cirrus sac and the vagina. The opening of the genital atrium is situated in the anterior third of each proglottis.

Male genitalia.—There are 30 to 36 testes arranged in a single row behind the ovary. Vas deferens is a very much coiled structure situated in about the middle of the segment. The coiled structure persists in the cirrus sac too.

Cirrus sac is an oblong structure, 220 μ long and passes beyond the excretory vessels. The inner excretory vessel and nerve cord pass over the cirrus sac while the outer excretory vessel passes below it. There is no specialized cirrus.

Female genitalia.—Ovary is a bilobed structure and each lobe is further composed of many smaller lobes. Behind the ovary is situated the vitelline gland. Anterior to the vitelline glands is the shell gland. From the shell gland is given off a fine duct which unites with the two ducts coming from each lobe of the ovary. From this point starts the oviduct which runs first anteriorly and then laterally. Near the genital atrium the oviduct becomes somewhat widened forming vagina, which opens in the genital atrium near the opening of the cirrus sac. Maximum diameter of the ovaries is 390 μ and of the vitelline glands 150 μ. Uterus is not distinct and receptaculum seminis is absent.

In the gravid segments the eggs are found scattered in the whole segment between the two outer excretory vessels. Ovaries and vitelline glands disappear first and then the testes and vast deferens with cirrus sac. Genital atrium however persists. Gravid segments are nearly one and a half time as long as broad.

Eggs are rounded structures about 42 μ in diameter. There is a thick-walled outer covering and a thin-walled inner membrane which contains the egg. Each egg is armed with 6 hooks, 16 to 18 μ long.

Discussion

About 21 species of *Oochoristica* have so far been reported from reptiles:—*O. tuberculata, O. amphisbæna, O. ameivæ, O. zonuri, O. crassiceps, O. trachysauri, O. lagrengi, O. thapari, O. parva, O. hainanensis, O. paravoria, O. bivitellobata, O. gracewileyæ, O. theileri, O. brasilensis, O. parvula, O. cryptobothrium, O. rostellata, O. khalili, O. fibriata,* and *O. Osheroffi*. Out of these, two *O. crassiceps* (Baylis) and *O. thapari* (Johri) have been reported from *Calotes*.

The species described in this paper can be compared with *O. parvula, O. thapari, O. trachysauri, O. theileri, O. crassiceps* and *O. tuberculata* to which it shows closer affinities but it can be readily distinguished from them all.

From *O. parvula* it differs in being much larger in size, by the absence of receptaculum seminis, and in having very much developed suckers. (The length of *O. parvula* is 20 to 25 mm. and the size of the suckers 170 to 190 μ.) From *O. thapari* and *O. trachysauri* it differs in size, the number of testes and their arrangement. (In both of these species the number of testes is about 60 while in the present case it is never more than 36.) From *O. thapari* it further differs in the size and course of cirrus sac, which in the case of *O. thapari* reaches nearly the middle of the segment and passes over both the excretory canals. From *O. crassiceps* and *O. theileri* it differs in having a larger number of testes, longer cirrus sac, well-developed suckers and distinct segmentation of the body. From *O. tuberculata*, which is nearly equal in size to the present form, it differs in having larger suckers (170 μ), a longer cirrus sac and in the number of testes. The number of testes in *O. tuberculata* range between 20 and 40 while in the present case it is never more than 36 or less than 30.

It thus appears to differ from all the known species of *Oochoristica* thereby justifying the creation of a new species, *O. indica*, for its reception.

Host.—*Calotes versicolor.*

Locality.—Lucknow.

Acknowledgement

I am deeply indebted to Dr. G. S. Thapar for the constant guidance and interest he has taken in this work. He made many useful suggestions and was very kind in placing his personal library at my disposal.

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AN EMBRYOLOGICAL STUDY OF *LINUM MYSORENSE* HYENE

By L. S. Dorasami and D. M. Gopinath
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Received May 23, 1945
(Communicated by Prof. L. S. S. Kumar, F.A.Sc.)

Literature on the embryological studies of the family Linaceæ is restricted to a few members of the genus *Linum*, particularly *L. usitatissimum*, *L. perenne*, *L. Flavum* (Schürhoff, 1924), *L. catharticum* (Souèges, 1924), etc. *L. mysorense* Heyne, which is indigenous to South India, grows in a wild state in marshy regions, specially on hill tops in association with species of *Eriocaulon*, *Lobelia* and others. A comparative account of the developmental stages of the female gametophyte and embryogeny of *L. mysorense* and some of the other species of *Linum* already known is presented in this paper.

Material for the present study was collected partly on the Nandi Hills, Mysore State, and the rest secured through the courtesy of Professor L. S. S. Kumar to whom our grateful thanks are due. For microscopic studies the material was fixed in Allen's modification of Bouin's fluid and formalin-acetic-alcohol. The sections were cut from 12-14 μ in thickness and stained in Heidenhain's iron-hæmotoxylin with eosin in clove oil as counter-stain.

Development of the Female Gametophyte

The ovary is superior and is 10 locular with a single anatropous ovule in each locule. The primordia for the ovule starts as a protuberance from the placenta and simultaneous with this the initials for the two integuments are formed. The initials for the obturator become differentiated early on the funiculus (Fig. 1). In later stages, the obturator composed of a pad of tissue arches over the micropylar end of the ovule. The archesporium is hypodermal and cuts off a parietal and a megaspore mother cell. Cases of occurrence of more than a single archesporium are met with (Fig. 2) but further development is confined to one archesporial cell only. There is a thin layer of nucellus surrounding the megaspore. As the embryo-sac enlarges in size following development, nucellar cells on the sides break down contributing nutrition, with the result the embryo-sac becomes lined with the epidermis of the inner integument. The rich cell contents and the presence of deeply staining nuclei in the epidermal cells point out the fact that the inner integument might well be considered as constituting the integumental tapetum. The megaspore mother cell undergoes the usual divisions (Figs. 3 and 4) and forms a linear tetrad of megaspores. The

division in the lower dyad cell is sometimes belated. The chalazal megaspore is functional and the rest degenerate (Fig. 5). The mature embryo-sac conforms to the monosporic 8-nucleate type.

The egg apparatus organised at the micropylar end consists of a small egg and two synergids which show conspicuous striations characteristic of filiform apparatus (Fig. 8). The antipodals degenerate by the time the egg apparatus is organised. The polars remain without fusion for a very long time (Fig. 7).

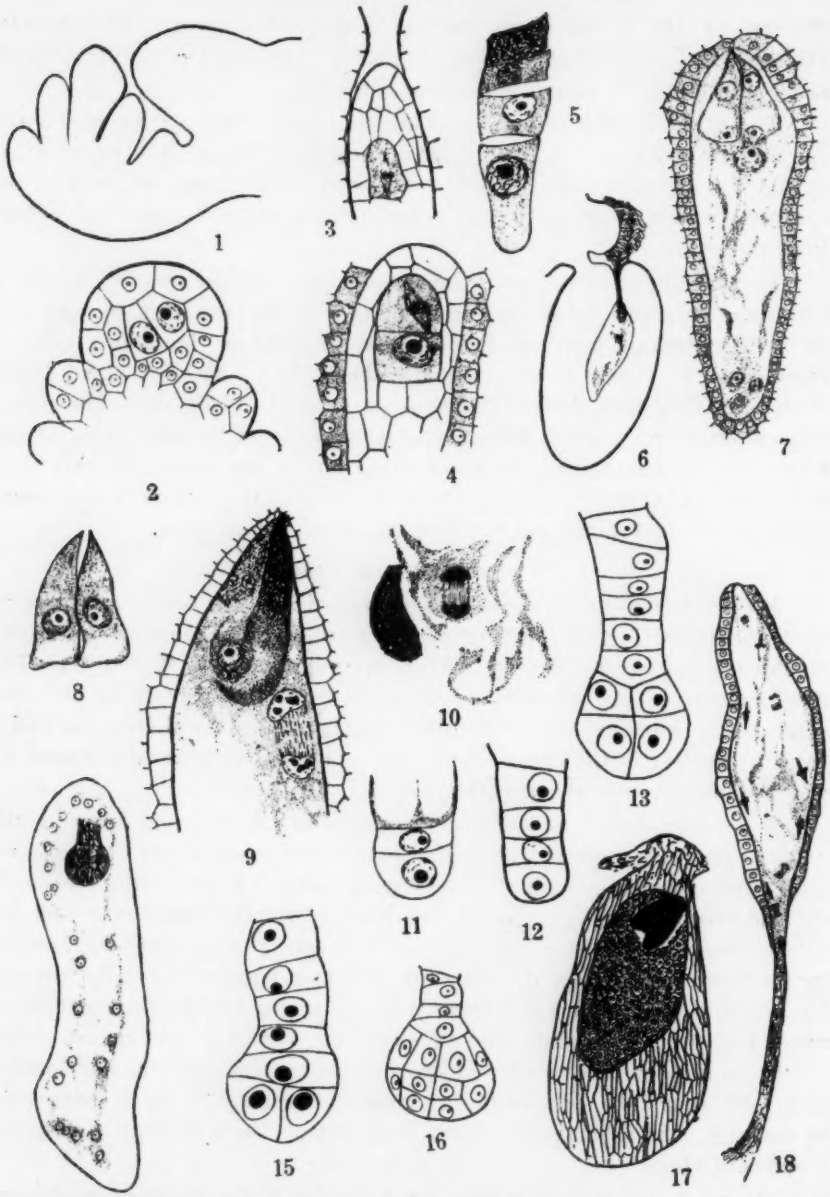
Fertilisation is of the porogamous type and the pollen tube, before it enters the micropyle is seen to grow along the obturator (Fig. 6), and it is likely the obturator performs the function of guiding the pollen tube as reported in the members of Labiatæ (Bushnell, 1936; Murthy, S. N., 1940), Araliaceæ (Gopinath, 1944), Thymeleaceæ (Kausik, 1940), etc. The pollen tube entering through the micropyle comes to lie on the membrane of the embryo-sac and during its passage crushes one of the synergids and coils round the egg (Fig. 9). Its tip bursts discharging the two male nuclei and triple fusion first takes place. Syngamy takes place later.

Post-fertilisation Stages

It is interesting to note that even before syngamy the primary endosperm nucleus divides (Fig. 9). When however fertilisation is complete the zygote divides transversely to form a 2-celled pro-embryo (Figs. 10 and 11). The development of the embryo is according to the capsella type. The suspensor is short and consists of 4-5 cells (Figs. 13, 15 and 16). The endosperm is nuclear in the initial stages (Figs. 14 and 18), later on followed by wall formation from the periphery.

Soon after fertilization is over, conspicuous changes take place in the ovule itself. The embryo-sac grows enormously towards the chalaza and establishes connection with the conducting strand of the ovule. Some of the endosperm nuclei are seen in this narrow passage which may well be called embryo-sac haustorium (Fig. 18). The tapetal cells which are formed by the inner epidermis of the inner integument persist but when the embryo has reached a fairly advanced stage they are encroached by the embryo-sac which has in the meanwhile grown considerably owing to the rapid divisions of the endosperm nuclei. When the endosperm assumes cellular nature at a later stage, the cells of the integumental tapetum distinctly noticeable in the early stages break down and degenerate. The obturator persists as a remnant (Fig. 17).

The most striking and interesting deviation in the developmental morphology of *Linum mysorense* from those of other species so far investigated



FIGS. 1–18.—Fig. 1. Development of the integuments and the initial for the obturator. ×570. Fig. 2. Archesporial cells. ×760. Fig. 3. Megaspore mother cell in metaphase. ×420. Fig. 4. Dyad; upper cell in division. ×760. Fig. 5. Linear tetrad of megaspores; upper

three degenerating. ×570. Fig. 6. Pollen tube growing over the obturator. ×80. Fig. 7. Embryo-sac with the full complement. Antipodals degenerating. ×440. Fig. 8. Synergids showing the filiform apparatus. ×900. Fig. 9. Fertilization of the egg and the first division of the primary endosperm nucleus. ×760. Fig. 10. First division of zygote. ×950. Fig. 11. Two-celled pro-embryo. ×570. Fig. 12. Four-celled pro-embryo. ×570. Figs. 13, 15 and 16. Stages in embryogeny. ×760. Fig. 14. Free nuclear endosperm and the embryo. ×570. Fig. 17. Condition of the ovule at an advanced statge in embryogeny. ×440. Fig. 18. Extension of the chalazal region of the embryo-sac into an haustorium at the post-fertilization stage. ×570.

is in the nature of endosperm formation. Schürhoff reports in the three species of *Linum* he has investigated, *viz., L. usitatissimum, L. perenne* and *L. Flavum* Heleobiales type of endosperm formation. In *L. mysorense* the development of endosperm is characteristically nuclear. The primary endosperm nucleus divides even before syngamy and the resulting two nuclei are not separated by wall formation (Fig. 9). In fact, there is no migration of any of the nuclei towards the chalaza. After the first division of the primary endosperm nucleus, it is followed by quick successive divisions resulting in the formation of free endosperm nuclei.

So far as the other features in the development of the female gametophyte are concerned, *L. mysorense* closely resembles the other species. Thus the type of embryo-sac development in *L. catharticum* (Souèges), *L. usitatissimum* (Schürhoff) and *L. mysorense* is monosporic 8-nucleate. The breaking down of the nucellar cells and the consequent formation of an integumental tapetum termed by Schnarf as "mantle layer" is also seen in *L. mysorense.* Prolongation of the chalazal end of the embryo-sac into an embryo-sac haustorium during post-fertilisation stages is common to both *L. mysorense* and *L. usitatissimum*. There seems to be a very close similarity in the developmental stages of the embryo in *L. catharticum* (Souèges) and *L. mysorense.*

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COMPARATIVE STUDY OF THE VEGETATIVE AND FLOWERING AXES OF *LEONURUS SIBIRICUS* L.

By Amiya Datta, M.Sc.

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Received February 22, 1945

(Communicated by Prof. G. P. Majumdar, F.A.Sc.)

Introduction

There is an ever-increasing volume of evidence that the cellular organization at the vegetative apex is fundamentally different from that at the inflorescence apex in angiospermous plants (Grégoire, 1935; Louis, 1935; Thompson, 1936; Foster, 1939; Majumdar, 1945). It has also been reported that there is a great difference in morphology, both external and internal, of the vegetative and flowering axes in many of them, particularly in the herbaceous species (Deats, 1925; Mirskaja, 1926; Wilton and Roberts, 1936; Graigner, 1943, and others). But the cause or causes responsible for these differences in the two axes of a flowering plant, are yet unknown, and a careful investigation is needed before an explanation can be offered.

Some work, however, has been done by plant physiologists to give a nutritional basis to flowering (Kraus and Kraybill, 1918; Nightingale, 1927; Finch, 1935; Roberts, 1936, and others), and attempts are being made to show the existence of a "positive correlation between the character of growth of a plant and its reproductive condition" (Roberts, 1936). Kraus and Kraybill (1918) suggest that when the ratio of carbohydrate to nitrogen rises above a certain value (?) the plants flower. Recent researches, however, do not uphold this theory, though it is admitted on all hands that a high value of carbohydrate nutrition is necessary for flower initiation.

It is a common knowledge that in many cases a land plant flowers more or less throughout the year. From this fact among others, Graigner (1943) considers that the flower initiation may be independent of the attainment of a 'photosynthetic maximum'; yet he maintains that a high carbohydrate supply should be guaranteed either by storage or by photosynthesis before a flower could be initiated, and the reason is quite obvious.

On the other hand, very little work, so far as the author is aware, has been done to find out the causal anatomy of the axes responsible for the production of the flowers. Graigner (1943) thinks that with the data at our disposal we can only state the problem, but not its solution. Wilton and

Roberts (1936), who made investigations on a number of herbaceous species to determine the relation, if any, between certain anatomical features in the axes and the production and non-production of flowers (p. 45), conclude that the differentiation of the structure of the flowering stem, precedes or accompanies, and is not the result of flowering, but both, *i.e.*, special anatomical features and flowering, are due to some physiological condition yet unknown.

In the Laboratories of the Presidency College a systematic study has been going on for some time past, on different types of shoot apex organization in herbaceous angiosperms. In *Leonurus sibiricus* L. a correlation between the reduction in the size and form of the leaf, branching and cambial activity and development of an endodermis in the axis and flowering, has been noticed. As these observations appear to the author to be interesting and perhaps made for the first time in this country, he considers it worth while to record them here.

The materials for the study were all collected from the plants growing in the College compound. Freehand and microtomed sections were made, and iodine and potassium iodide solution, chlorozinc iodine, phloroglucin, safranin, Delafield's hæmatoxylin, safranin and fast green combinations were tried with equal success. All figures with the exception of Text-Fig. 1, are drawn under camera lucida at magnifications given in the explanations.

Morphology (Fig. 1)

Leonurus sibiricus L. (Family Labiatæ) is an annual weed growing in waste places; the square stem is erect, stout and grooved, and is normally 2–6 feet high. Stem and leaves are covered with hairs and glands. At the time of flowering the vegetative shoot continues into the flowering one. The leaves are 2–4 inches in length, exstipulate and are arranged in opposite decussate phyllotaxis. Long slender petiole is grooved throughout the entire length on the adaxial surface. The incision of the leaf-blade is a characteristic feature of the plant and it shows a progressive variation from an almost entire palmate blade in the seedling stage, to toothed, lobed and multisected ones in the vegetative region; finally the blade becomes reduced to a single linear lanceolate piece at the few crowded nodes near the apex of the flowering shoot. Lower leaves are reticulately palmate veined, but they are uniseriate pinnate near the apex in the floral region. The adult stem is naked for the few lower nodes by the early fall of leaves at these nodes.

Branching which is confined to the 4–6 upper nodes of the vegetative region, is conspicuously absent in the flowering region. But in case the

flowering point is injured in the early stage of growth and development, branches are developed at the first and second nodes near the base, and the plant assumes a bushy appearance, as is often seen in the field.

The first few nodes are very short and woody, the next four or five are much longer in comparison with the lower ones and show much vegetative growth. After this, particularly in the flowering region, the internodes decrease in size, the shortest ones being noted near the tip. The leaves are also seen to vary in size and area according to their position on the axis, the lower being bigger than the one next above it.

Leonurus sibiricus is a plant of short duration, does not live for more than 3 or 4 months, but there is a constant succession all the year round. It generally flowers at the commencement of the rainy season, but also more or less throughout the year.

Anatomy (Figs. 2*a*–4*b*)

The structure of the innermost layer of cortex and vascular bundles is described here. Detailed anatomy of the stem is given elsewhere.

Starch Sheath and Endodermis.—In the seedlings and young plants this layer is wholly in the starch sheath condition, *i.e.*, with abundant starch contents, but with no Casparian thickenings on the radial and transverse walls of its cells (Fig. 2*a*). In the adult axis this layer is easily distinguished as a single layer of thin-walled rounded parenchymatous cells densely packed with starch grains. The Casparian strips are not developed at all. Near the upper end of the vegetative region, these thickenings are seen just appearing on the radial and transverse walls indicating that the vegetative axis will soon enter into flowering condition. This stage has been described by Priestley and others as the primary stage of the endodermis (Priestley, 1922; Soar, 1922; Warden, 1935). With the appearance of the Casparian thickenings the starch grains begin to disappear.

In the flowering region, which is a continuation of the vegetative region, a true endodermis is developed. The cells of this layer thickens more and more till the whole of the radial and transverse walls are covered with Casparian thickenings. This is the secondary condition of the endodermis according to Priestley and others (Fig. 2*c*–2*e*). The tertiary condition, which means the enveloping of all the walls with secondary thickenings, is not found in *Leonurus*. The starch sheath is crushed when secondary growth has proceeded in the vegetative axis for some time.

Vascular Bundles.—In the vegetative axis four synthetic bundles, each opposite a ridge, and one to three cauline secondary bundles, opposite each

furrow, are disposed of in a ring. The cambium is very broad and continuous through these bundles, and shows much activity.

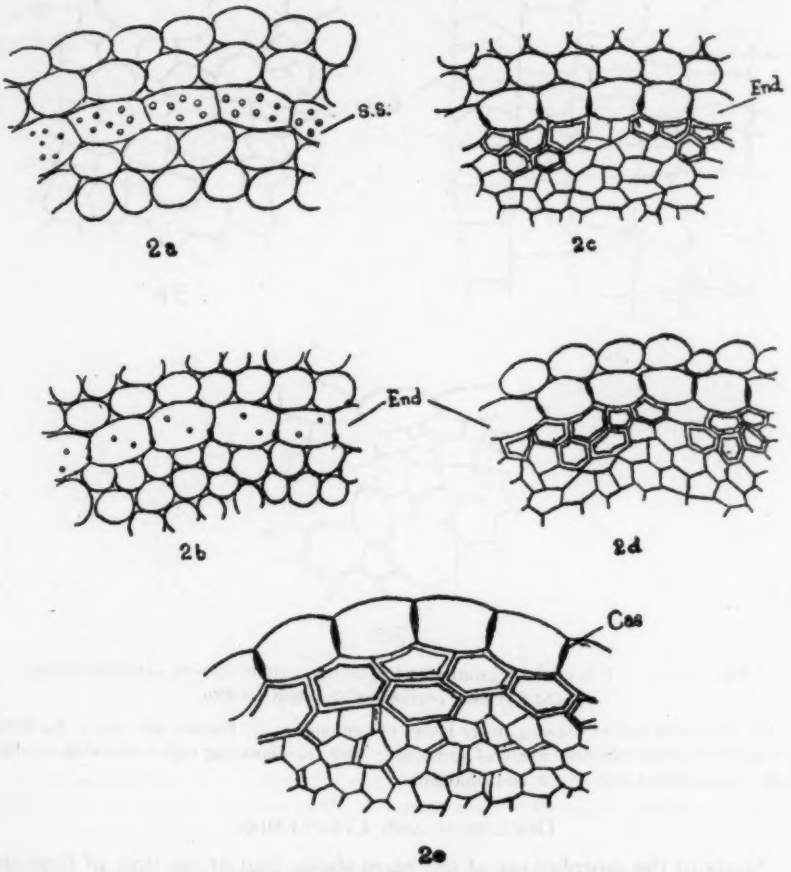

FIG. 2 (*a–e*). T.S. of the portion of the stem showing starch sheath and endodermis at different stages of development.

(*a*) Starch sheath (*s.s.*) in seedling and young plants (×450); (*b*) Primary condition of endodermis in adult vegetative plant; Casparian thickenings (*Cas*) appearing on the radial walls, starch is still present (×350); (*c–d*) Secondary condition of endodermis (*End*) in flowering plants; Casparian thickenings cover whole of the radial and transverse walls, starch completely disappears (×350); and (*e*) Endodermis in mature flowering plant (×550).

In the flowering axis the cambial activity is exhausted soon, and is hardly, if at all, distinguishable, being completely differentiated into xylem and phloem (Figs. 3*a* to 4*b*).

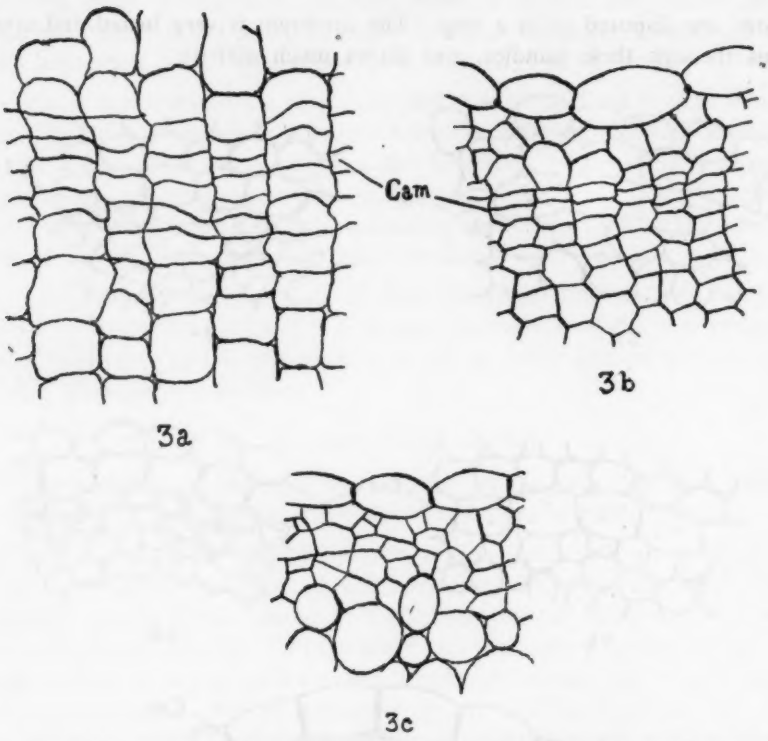

FIG. 3 (*a–c*). T.S. of the vegetative and flowering regions showing cambium (*Cam*) in the furrowed portions of the stem (×400).

(*a*) Vegetative region showing many layers of cambium; (*b*) Portion just below the flowering region showing only few layers of cambium; and (*c*) Flowering region showing cambium wholly differentiated into xylem and phloem.

DISCUSSION AND CONCLUSION

Study of the morphology of the plant shows that at the time of flowering the vegetative axis continues direct into the flowering shoot and the branching which is confined to the vegetative region is conspicuously absent in the flowering region. In the latter the leaf-blades become reduced in size and their incisions more and more deeper till finally it becomes reduced to a single linear-lanceolate piece at the apex.

The extreme reduction in the leaf-surface and the absence of vegetative buds in the flowering region is correlated with the development of the endodermis with distinct Casparian thickenings. The Casparian strips place he exchange of water and solutes through the primary endodermal cylinder

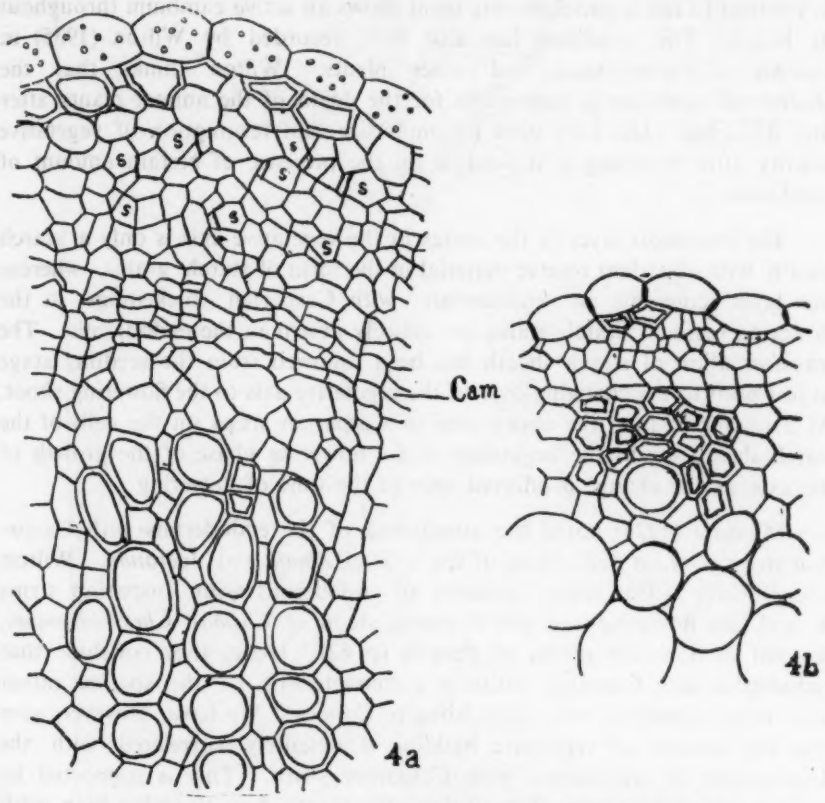

FIG. 4 (*a-b*). Showing vascular bundles in vegetative and flowering regions in T.S.

(*a*) Vascular bundle in the vegetative region showing cambium between xylem and phloem (×400); and (*b*) Vascular bundles in the flowering region showing the absence of cambium between xylem and phloem (×480).

under the control of living protoplasts (Priestley and North, 1922, p. 125). Miss Soar ascribes this factor as contributory to the xeromorphy of the leaf of Abietineæ (1922, p. 290).

When the anatomy of the vegetative and the flowering axes is compared two interesting facts are observerd: difference in the cambial activity and the behaviour of the innermost layer of the cortex in the two regions. In the vegetative stem, both in young and adult plants, the cambium is well represented, being a many layered meristem, whereas in the flowering region there is no organised cambium. Furthermore, there is little or no cambial activity in any part of the stem of an abundantly flowering plant of *Leonurus*.

In contrast to this a non-flowering plant shows an active cambium throughout its length. This condition has also been recorded by Wilton (1935) in *Cosmos, Chrysanthemum* and other plants. Wilton thinks that the absence of cambium is responsible for the death of the annual plants after first flowering. She even goes to emphasize that resumption of vegetative activity after flowering is dependent on the presence of certain amount of cambium.

The innermost layer of the cortex in the vegetative axis is only a 'starch sheath' with abundant reserve material in the form of starch grains, whereas this layer represents an ' endodermis ' with Casparian thickenings in the flowering region. Starch grains are entirely absent in the endodermis. The transformation of starch sheath has been followed from the seedling stage to just prior to the changing-over of the vegetative axis to the flowering shoot. As a matter of fact, the appearance of Casparian strips on the cells of the starch sheath marks the beginning of the flowering phase of the growth of the axis, as the change is effected only at the time of flowering.

Mirskaja (1926) noted the appearance of the endodermis with Casparian strips only on disbudding of the axes of *Zinnia* and *Ageratum*. Wilton and Roberts (1936) report presence of endodermis with Casparian strips in both the flowering and non-flowering stems of *Cosmos, Chrysanthemum*, but not in flowering plants of *Petunia* (p. 62). Hence they conclude that endodermis with Casparian strips is a characteristic of the species rather than being associated with disbudding in *Cosmos*. We have, however, seen that the absence of vegetative budding is definitely correlated with the development of endodermis with Casparian strips. This is supported by the occurrence of occasional small flowering plants, 8 to 10 inches high, with conspicuous endodermis with Casparian strips.

All these facts show that the absence of branching, reduction in leaf size, early cessation of cambial activity and development of a typical flowering condition of axis go together. The cessation of cambial activity and the development of an endodermis may be responsible for the non-production of vegetative buds and size reduction of the leaves. This may cause a physiological unbalance in the plant by reducing its photosynthetic activity. This may stimulate the production of flowers, but what causes the development of the Casparian thickenings on the endodermal walls and cessation of cambial activity is yet unknown. From the data at our disposal it cannot be ascribed to chronological age of the plant. On the other hand, an experiment coducted in the Laboratory on water culture of Maize, showed that the production of flowers could be forced by starving the plant. And we have seen in *Leonurus*

FIG. 1

Adult plant with flowers. Also different sizes and shapes of leaves are drawn according to their positions on the stem.

that the development of a typical endodermis is responsible for the reduction of food manufacture in this plant.

Summary

In the study of the anatomy of the vegetative and flowering regions of *Leonurus,* three important features have been observed:

1. The unbranched condition of the flowering axis with extreme reduction of leaf lamina;

2. The greater activity of cambium in vegetative than in the flowering region which appears to be due to the difference of photosynthetic activities in the two regions of the plant; and

3. The endodermis which is well developed in the flowering region is only a 'starch sheath' in the vegetative region. The change from starch sheath to endodermis appears to be concerned with the xeromorphy of the flowering region indicated by the extreme reduction of the leaf lamina in this region.

The author wishes to express his deep sense of gratitude and indebtedness to Prof. G. P. Majumdar for his valuable help and guidance in the course of investigation.

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COMPARATIVE MORPHOLOGY OF NORMAL MAMMARY GLANDS OF FOUR STRAINS OF MICE, VARYING IN THEIR SUSCEPTIBILITY TO BREAST CANCER*

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Received February 24, 1945

(Communicated by Dr. V. R. Khanolkar, F.A.Sc.)

Cancer of the breast has occupied the attention of investigators for a long time. The clinicians and pathologists have been mainly concerned with the disease as it occurs in human subjects and have attempted to correlate the course of the disease with heredity, child-bearing, histological structure and other factors. The discovery of the occurrence of spontaneous mammary cancer in laboratory animals has opened a new method of approach to the problem. The scope of investigations has been broadened with the rearing of pure homozygous strains of mice, some very highly susceptible to breast cancer and others relatively insusceptible. The investigations of Allen, Leo Loeb, Murray, Gardner, Strong and others have cleared many obscure places in an understanding of the development of this disease in mice. It appears that an almost quantitative relationship exists between the origin of cancer and an interaction of the internal secretions which initiate or sustain continuous or periodic growth processes in the body, and the genetic or hereditary factors. Leo Loeb[16] (1941) has attempted to express this idea in the form of an equation S (hormonal stimulation) × H (Heredity) = C (Cancer). Since the early experiments of Lacassagne[15] (1932–33), on production of mammary cancer by repeated injections of estrogenic substances, the effect of ovarian and other hormones in normal and castrated animals has been extensively studied. [Zondek[23] (1935–40); Gardner, Strong and Smith[9] (1935); Burrows[6] (1935); Miller and Pybus[18] (1942); Biskind and Mark[1] (1939); Taylor and Waltman[19] and others]. It has recently been discovered by Bittner[2,3] and confirmed by others that certain substances or properties in the milk on which the young animals are reared affect the susceptibility of mice to breast cancer. These substances or properties have been shown to be present in the different organs as well as in the milk and are transferred during the process of suckling.

*This study forms a part of the thesis for Ph.D. degree of the Bombay University. The work is at present in progress at the Tata Memorial Hospital under the guidance of Dr. V. R. Khanolkar.

At the Tata Memorial Hospital, investigations have been in progress for the last three years on a study of the metabolism of different strains of these mice. Khanolkar and Chitre[13] have shown that the esterase activity of the serum appears to alter with the susceptibility to cancer, and that the milk factor effect seems to be reflected in the quantity of serum esterase. During the course of this investigation it was suggested that a careful morphological study of the mammary glands in these experimental animals may supply useful information regarding the early structural differences which lead to a later development of breast cancer. This idea was supported by the observations of Gibson[12] (1930) that the "predisposition to tumour incidence might be determined or indicated by structural or developmental factors." Her observations further suggested that the mammary glands of susceptible strains generally develop less rapidly than those of a resistant strain. Before undertaking a detailed morphological study of the mammary glands in susceptible and insusceptible strains of mice subjected to various experimental procedures, it was necessary to acquaint ourselves with the normal development of the glands at different age periods. This paper deals with the preliminary work which extends the observations of previous investigators and fills in certain gaps which were revealed after a careful study of the available literature on the subject. A brief review of the previous work would not be superfluous at this stage.

The normal development of mammary gland of mouse has been extensively discussed by Turner and Gomez[20] (1933) and by H. A. Cole[7] (1933). Cole describes the development of mammary gland during all phases of sexual cycle, during pregnancy, lactation and the regression which follows it. He has however not studied pure strains of mice, neither has he given any comparison of susceptible and resistant strains. Gardner and Strong[10] (1935) have described the comparative morphology of mammary glands of different strains of virgin mice, varying in their susceptibility to breast cancer. They have compared the glands of ten strains from 25 to 100 days of age at intervals of 6 to 8 days. They did not notice any structural difference between glands of various strains upto this early age of 100 days. E. Fekete[8] compared the mammary glands of two strains C57 (0·5 per cent. susceptibility to breast cancer in breeding females) and *dba* (55 to 60 per cent. susceptibility). She studied the glands of females from one week old to four weeks, at weekly intervals and did not report any morphological difference between the virgin mammary glands of the two strains. She has further studied the comparative morphology of mammary glands of the same strains during pregnancy, lactation and regression for four consecutive pregnancies. Gardner, Strong and Smith[11] (1939) studied the glands of adult females of five different strains

at different post-lactation intervals. They reported a frequent occurrence of a number of localised nodules in the glands of the high tumour strains which were very rare in the glands of the low tumour females. Loeb and Suntzeff[17] (1941), in their recent study of 'growth processes in mammary glands of seven different strains', have collected the data from 756 different animals including few castrated males and females who were administered subcutaneous estrogen injections. They concluded that various strains do differ in the average rapidity with which their glands grow on stimulation with estrogens. Taylor and Waltman[19] (1940) appear to be the first to study these virgins at later age. In their publication on 'hyperplasias of the mammary gland in the human being and in the mouse', they have made only a passing reference to these virgins of C57 and *dba* strain between the ages 2 to 6 months. They report a definite difference as regards the number of ductal branches and acini (hyperplastic nodules) in the glands of two strains as early as 2 to 3 months, and that this difference is completely established by the age of 6 months. van Gulik and Kortweg[21] confirmed the same observations and believed the hyperplastic nodules to be associated with active milk agent.

The above review of previous literature shows that many investigators have studied the morphology of the breast but some have failed to use homozygous strains and others have not followed the changes that supervene at definite age periods. The present investigation was planned to collect information which was lacking so far in the work of previous investigators.

Method and Material

Four highly inbred strains of laboratory bred mice were used for the experiment:—

1. C_3H.—(95 per cent. susceptibility to spontaneous cancer of the breast).

2. A.—Small albino mice (80 per cent. suceptibility in breeding females and 4 per cent. susceptibility in virgins).

3. *dba*.—Little's *dba* (55 per cent. susceptibility in both breeding and non-breeding females).

4. C57.—Black (0·5 per cent. susceptibility in breeding females looked upon as the strain resistant to cancer).

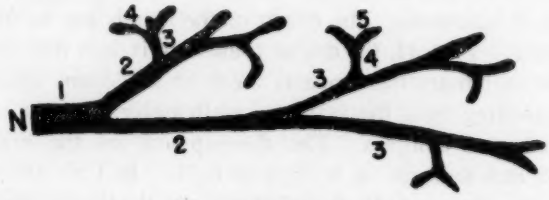
92 Virgins of the four strains were sacrificed, 60 between the ages of $2\frac{1}{2}$ to 6 months at bi-weekly intervals and 32 in the age groups 6 to 8 months at monthly intervals. Four females of each strain were killed between the age of $2\frac{1}{2}$ to 3 months and at the age of $5\frac{1}{2}$ to 6 months. So that on an average 3 pairs of mammary glands of 15 virgins of each strain upto the age of 6 months and of 8 females of each strain at the later age of 6 to 8 months were available for study.

Daily vaginal smears were taken, and care was taken to see that every virgin was killed at definite estrus, as the structure of the mammary gland changes with phases in the sexual cycle (Cole[7]). The animal was killed with chloroform and flaps of skin bearing the mammary tissue were cut, stretched and stitched on tiles that were flooded with 25 per cent. formalin, or Bouin's fluid. From the skin thus fixed overnight, entire breasts were dissected next day according to the technique suggested by Gardner, Strong and Smith[11] (1939). Permanent gross mounts of the first 3 pairs of mammary glands were stained with Kernechtrot and used for the morphological study. The first gland on the right side of every animal designated R. was always used for the entire sketch with the help of a camera lucida. Portions of the glands showing remarkable cell proliferations were usually cut and preserved in weak alcohol for paraffin sections.

Observations

I Age-group: 75 *to* 95 *days*.—Mice generally reach maturity at the age of 50 to 55 days. Virgins of different strains studied soon after maturity (*i.e.*, at the age of 75 to 95 days) showed some minor differences in the structure of the mammary glands. On comparing R_1 (first thoracic gland on the right side) of different strains at this age it is seen that the general plan of the mammary glands is the same in all the four strains.

R_1 of every strain consists of a big dilated primary duct which opens externally through the skin. There are two main secondary branches. Each one of the secondary branches gives off tertiary branches and occasionally branches of the fourth order. The ramification of one of the secondary ducts generally extends in a plane at right angles to the main primary duct, while the other secondary duct produces two long slender tertiary branches extending almost the whole length of the gland which further give off side branches of the fourth and fifth order (Text-Fig. 1). Although the general plan of the main big branches of the mammary tree is more or less similar



Text-Fig. 1

in all the strains; a careful study of the details of surface extent of glands and ductal branching reveals few remarkable differences.

Out of four virgins of C_3H studied at the age of 75 to 95 days three show a similar pattern of ductal branching upto fourth order, with few side branches and few acini. The remaining animal, almost an exceptional case, has glands showing a considerably larger number of side branches and acinar clusters. There was thus an evidence of a certain amount of individual variation between members of the same strain in the same age group. On comparing the normal glands of C_3H with that of A it is seen that the glands of A show a larger surface spread and greater complexity of ductal branching. The glands of the third susceptible strain, *viz.*, *dba*—show a structure very different from that of either C_3H and A. In *dba* the primary and secondary ducts are much more dilated, the lumen of the ducts is filled with some sort of secretion and the ductal branching is comparatively simple. Branches of the fourth order are rarely seen and the mammary tree covers a smaller area. This simple structure of the glands in *dba* strain does not resemble the structure in C_3H and A. The glands of the non-susceptible strain C57 show a morphological structure similar to that of *dba*, with dilated main ducts, few side branches and small surface spread of the gland [Plate II, Fig. 1 (1 to 4)].

According to Taylor and Waltman[19] (1940), 'the main ducts of *dba* are similar to that of C57 at about 90 days possibly more slender; and the terminal branches occurring at the sides and ends are definitely more numerous'. The above observations in the four strains show, that differences in the extent of glands, number of side branches and dilatation of ducts exist between the susceptible and non-susceptible strains at the age of 3 months—though not between C57 and *dba*. The *dba* strain appears to form a class by itself though simulating more the structure in C57 than the other two strains.

II Age-group: 120 *days*.—In the virgins studied at the age of four months the ducts are found to grow peripherally from terminal buds and new branches develop from buds forming along the walls of the ducts in all the different strains. The differences that were visible at the early age of 95 days have become more conspicuous. The extent of the glands has naturally increased in all the strains. In C_3H, the ductal branching is seen only upto the fourth order and the mammary tree appears more or less open, while in strain A, there is a branching upto the fifth and sixth order, and the mammary tree appears closed and complex. The development of the mammary gland is more rapid and uniform in A than in C_3H. In C57, the surface extent of the gland is less than in C_3H, branching is upto the third order but dilatation of main ducts is not so conspicuous as it was at 95 days.

III Age-group: 120 *days to* 135 *days*.—In a period of two weeks, *i.e.*, from the age of 4 to $4\frac{1}{2}$ months, very little further growth of mammary glands occurs

except for the slight cyclic hypertrophy of ducts with each estrus. Ultimately at the age of 4½ months the strain A shows a conspicuously closed and complex gland, the C_3H, a comparatively simple and open structure with few side branches and C57 very similar to that of A.

IV Age-group: 150 *days.*—So far there were only minor morphological differences as regards the number of side branches, surface extent of glands, dilatation of ducts, etc., but at the age of five months some interesting developmental strain differences begin to unfold themselves. Most of the glands of C_3H studied at this age show conspicuously slender ducts, simple branching and occasional acinar clusters. Acini in certain regions of the gland multiply more rapidly than in others. Few glands of A also show acinar clusters at this age, but the acinar multiplication is remarkably uniform along the sides and at the ends of ducts in strain A. The glands of C57 are conspicuous by an absence of acinar clusters. They show simple branching and slender ducts. The glands of the susceptible *dba* strain studied at this critical age, show a peculiar architecture. The two main secondary ducts of R_1 are stunted in growth, the branching is exceptionally simple with few small branches of the third order, and there are short, blunt processes all along the sides of ducts. All the ducts have wide lumina and the area covered by the mammary branches is the least extensive of all the glands studied so far.

V Age-group: 150 *to* 165 *days.*—It is generally at the age of 5½ months that a definite developmental difference between mammary glands of strains of varying susceptibility is first observed. An examination of R_1 of C_3H virgins shows small projections all along the sides of secondary and tertiary ducts [Pl. II, Fig. 2 (1)]. In R_3 (third thoracic gland on the right) of the same animal a focal proliferation of cells is clearly visible (Pl. III, Fig. 5). The ducts are slender, the branching is simple but few clusters of proliferating cells seen at ends and sides of ducts are conspicuous even under the dissecting lens ($\times 6$). The clusters appear to result from an irregular growth from certain cells of the ducts or acini. The glands of the strain A show ductal branching very similar to that in C_3H—probably a little bit more complex—but the focal proliferation of cells seen in C_3H is completely absent. In the non-susceptible C57, the ducts are a little more dilated than in C_3H and A, branching is simple —there are few side branches and no foci of proliferation. The morphological picture of mammary gland of C57 at this critical age is much simpler than that of C_3H and A. The architecture of the glands of *dba* does not resemble that of any other strains. The increasing age of the animal has only widened the ductal passages and has extended the length of the gland to a certain extent. It has not added to the complexity of ductal branching. The branching is still simple with a large number of short blunt processes all along the

sides of the ducts. It can be definitely stated that apart from the strain *dba* (little) which develops in a special manner, the other three strains show a significant developmental difference in the structure of the mammary glands at this age of 165 days [Pl. II, Fig. 2 (1 to 4)].

VI Age-group: 180 *days*.—The significant proliferative difference that makes its first appearance at the age of 5½ months is completely established by the age of 6 months. In C_3H female, 185 days old, R_1 shows ductal branching upto the fifth order [Pl. II, Fig. 3 (1)]; ducts are slender and the gland is comparatively a closed one while the focal proliferation of cells appearing at ends and sides of ducts in both R_1 and R_3 is more conspicuous and much better developed than at 5½ months. Microscopical examination of sections cut parallel to the skin surface of inset in [Pl. II, Fig. 3 (1)], shows tubular ducts with basement membrane surrounded by adipose tissue. The ducts are lined by two layers of epithelial cells, a basal and an inner layer of cuboidal or polygonal cells with indefinite cytoplasm. Nuclei are round or ovoid in shape and mostly uniform in size. Acini (the end bulbs) are lined by two or more than two layers of similar epithelial cells and the clusters are composed of groups of cells derived from the epithelial lining of the ducts as a result of intensive proliferation at certain focal points (Pl. III, Figs. 6 and 7). Taylor and Waltman[19] using *dba* as the susceptible strain in comparison with the non-susceptible C57—has noted a structural difference as regards the number of acini and side branches—but they do not report any proliferation of epithelial layers.

Comparing the glandular morphology of C_3H with that of the strain A at 6 months—it is found that the gland of A is more closed and complex; besides there are uniform acinar clusters all along the sides and ends of ducts [Pl. II, Fig. 3 (2)]. The acinar clusters of A have a definitely different appearance in gross preparations than those of irregular focal clusters in C_3H [Pl. II, Fig. 3 (1)]. Acinar clusters in the strain A are merely formations of new acini for uniform rapid growth of the gland, but the focal proliferation in C_3H is definitely a beginning of some irregular growth in selected regions of the gland—that might lead to a pathological change in later life. The non-susceptible C57 shows simple branching upto the 3rd order of slightly dilated ducts, very few side branches and no hyperplastic nodules. The difference in the morphology of the non-susceptible and the two susceptibles C_3H and A is clearly established.

All the four virgins of *dba* killed at the age of 6 months give a remarkably uniform picture of mammary glands. Breadthwise they cover the smallest area possible, the ducts are about 4 times more dilated than those of C_3H

and A and branching is simple upto the third order with few short blunt processes along the ducts. The picture differs from every other picture of different strains studied so far [Pl. I, Fig. 3 (1 to 4)].

VII Age-group: 180 *days to* 210 *days*.—After finding out the exact age at which the significant structural differences in mammary glands of different strains are clearly manifested, same sort of comparative study of the glands was continued for two months more.

The study of breasts of different strains at the age of 6 to 7 months reveals little advance in the development of the glands of all the four strains. Four out of six virgins of C_3H show focal proliferations of cells at the ends and sides of ducts at few places, while in two out of three virgins of A there are uniform acinar clusters all along the ducts indicative of rapid development of the glands. Two out of three females of C57 show simple branching upto the fourth order with few side branches while four out of five virgins of *dba* show peculiarly dilated ducts with short, rounded processes along the whole length of the ducts. The above description shows that morphological sketches of glands of four strains at six months (Pl. II, Fig. 3) are equally representative of the glands at seven months.

VIII Age-group: 210 *days to* 240 *days*.—During the period of 7 to 8 months the morphological differences appear to develop more or less rapidly and they show a more advanced stage in all the four strains.

Out of five virgins of C_3H, three show well differentiated focal proliferations [Pl. I, Fig. 4 (1)]. The remaining two females show simple ductal branching. This may be due to individual variation in the strain. It has also to be borne in mind that although the animals belong to a susceptible strain every animal does not develop the carcinoma of the breast; nor does the cancerous process commence at an early age in all members of the same strain. The proliferations in the three virgins are at a pretty advanced stage in the thoracic pair of larger mammary glands [Pl. II, (Insets) Fig. 4 (1)]. They are seen at more than six to seven points. Another feature which was observed was an occasional dilatation of the ducts in connection with proliferating cells in C_3H [Pl. II, Fig. 4 (1)]. The ductal branching is little more complex than at the age of six months. Comparing the glandular morphology of C_3H with that of A the branching in A is seen to be more extensive and complicated. It might however be pointed out that in a batch of four virgins of this strain none shows the uniform acinar clusters usually so common in the strain A. The focal proliferations are conspicuous by their absence. Three out of four virgins of C_{57} give a uniform picture with simple branching slightly more complicated than at 6 months and no cell proliferations. The

fourth one shows a pattern somewhat similar to that of A with uniform acinar clusters. *Dba* has nothing special to exhibit except its dilated ducts, simple branching and short round projections all along [Pl. II, Fig. 4 (1 to 4)].

Discussion

The detailed morphological study of mammary glands of 92 virgins of four different strains ranging in age from 75 to 240 days (2½ to 8 months) has clearly established certain significant developmental differences in different strains. The minor differences as regards the dilatation of ducts, number of side branches and acini in the mammary tree are visible in the strains C3H, A and C57 as early as 2½ to 3 months. There are also noticed the significant focal proliferations indicative of abnormal growth at a later age in C_3H and their absence in the remaining three strains at the age of 5½ to 6 months and later at 6 to 8 months. A general statement to this effect has been made by Taylor and Waltman[19] and also by van Gulik and Kortweg[21] · unaccompanied by any precise description. Besides these significant gross differences in the morphology of different strains there are certain points regarding individual strains, that need attention at this stage.

The observations as regards different strains at different age periods show that there is individual variation in all the strains. It is least in the strain Little's *Dba*, most in the C_3H and almost to a similar degree in the strains A and C57. Glands of *dba* show remarkable uniformity in their structure which is rarely seen in the other three strains.

The age—5½ to 6 months—at which the focal proliferations indicative of later abnormal growth make their first definite appearance in the susceptible strain C_3H—appears to have some significance. Studying the incidence of breast cancer in this particular strain according to age groups it is found that the earliest age at which the virgins are seen to develop breast tumours is about the 4th month and the maximum number of virgins develop tumours at the age of 8 to 11 months (Khanolkar and Chitre[14]).

Careful study of the individual strain A reveals that though A is supposed to be one of the strains highly susceptible to spontaneous carcinoma of the breast, out of 25 virgins studied none shows focal proliferations in mammary glands indicative of late abnormal growth. This absence of hyperplastic nodules in the virgins may be indicative of an exceptionally low incidence of cancer in virgins—only 4 to 5% as against 80 to 85 per cent. in breeding females (Bittner[4]).

Further, the glands of A develop more rapidly, branch profusely and the mammary tree is more closed and complicated than that of C_3H, all

along from 2½ to 8 months with few exceptions. The rapid development brought about by acinar clusters is always uniform in all the regions of the gland. It is because of this uniformity in development that though the glands are even more extensive than of C_3H—the incidence of cancer in the virgins of A, is almost negligible compared to that in the virgins of C_3H.

The observation regarding the uniform extensive branching in strain A and its difference from that of C_3H has been previously reported by Bittner and others[5] and also by Visscher and others.[22] Bittner believes that 'spontaneous mammary cancer in mice will develop only in glands which have been stimulated to growth by estrogenic harmones'. In both C_3H and A the glands develop rapidly but they are without hyperplastic nodules in the strain A; this indicates characteristic difference in hormonal metabolism in mice of two strains. There is either a difference in the amount of estrogen produced or the sensitivity of mammary structure to estrogens or a combination of both these factors. These differences may indicate the increasing susceptibility to cancer (80 per cent.) after repeated pregnancies.

As regards the susceptible strain *dba*, it is difficult to explain the reason for a complete difference in appearance from that described by E. Fekete[8] and Taylor and Waltman[19] who have used the same strain as a highly susceptible one. The structure seen in all the virgins of *dba* so far studied differs completely from the other susceptible strains C_3H and A. Focal proliferations indicative of late abnormal growth were not observed in this particular strain and it is difficult to explain the high incidence of breast cancer in this strain on the basis of breast morphology. These findings suggest that a susceptibility to spontaneous carcinoma of the breast may probably be dependent on factors which are not identical in the different susceptible strains.

Conclusion

1. The detailed study of comparative morphology of mammary glands of four different strains C_3H, A, *dba* and C57—from the ages of 2½ to 8 months shows that certain minor differences as regards the number of side branches, acinar clusters and dilatation of ducts are seen as early as 2½ to 3 months.

2. The significant developmental differences leading to abnormal growth at a later age are exhibited at the age of 5½ to 6 months between the strains C_3H, A and C57 while *dba* forms a class by itself. The structural peculiarities of different strains are seen at definitely advanced stage at a later age of 6 to 8 months.

3. An attempt has been made to discuss the bearing of morphological differences on strain susceptibility to cancer.

ACKNOWLEDGEMENT

The author is indebted to Sir Dorabji Tata Trust, for the grant of a research scholarship which has enabled the above investigation. Thanks are due to Mr. R. V. Nerurkar and S. A. Imam for technical assistance.

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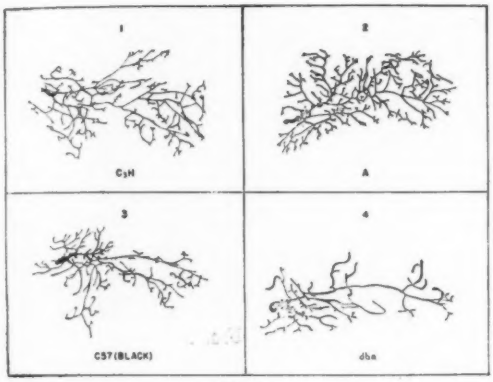

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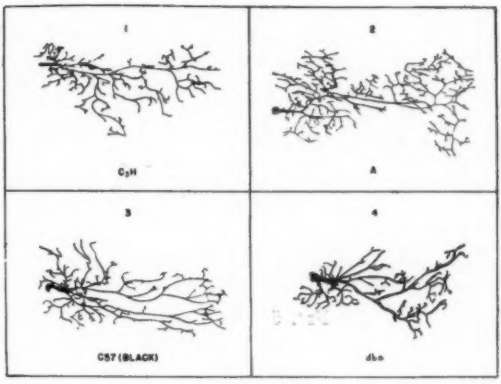

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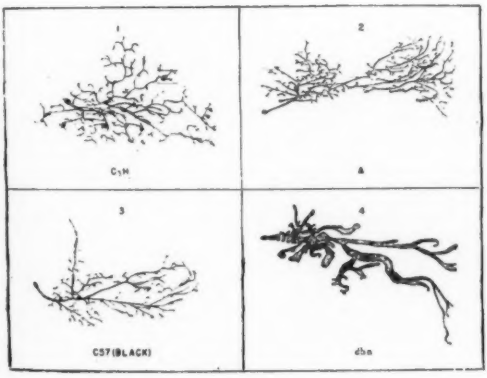

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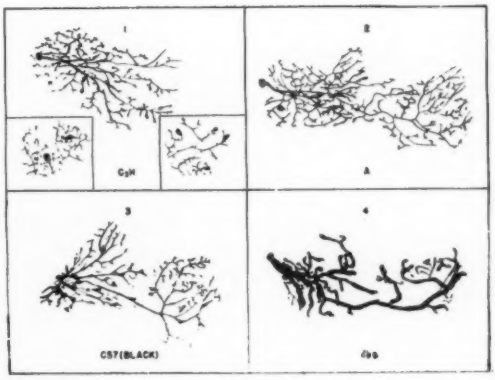

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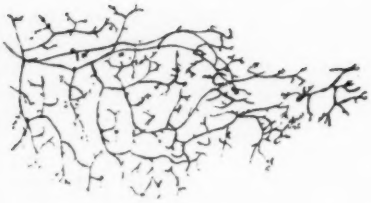

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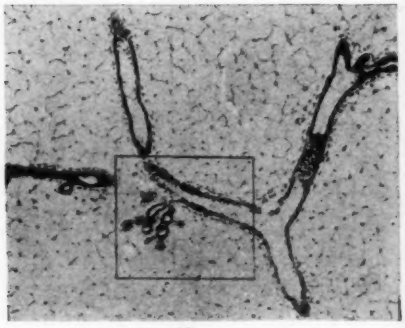

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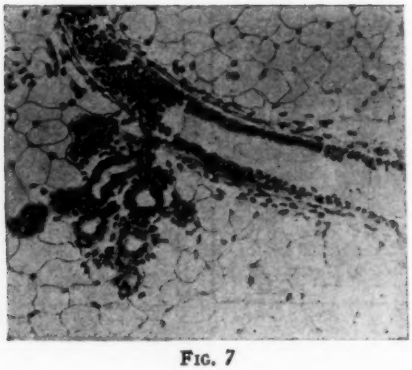

FIG. 7

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Explanation of Plates

Plate II

Fig. 1. Silhouette of the first thoracic mammary gland on the right side of virgin mice of the C_3H, A, C57 (Black) and *dba* (Little) strains 75 to 90 days old. Enlarged two times.

Fig. 2. Silhouette of the first thoracic mammary gland on the right side of virgin mice of the C_3H, A, C57 (Black) and *dba* (Little) strains 165 days old. Enlarged two times.

Fig. 3. Silhouette of the first thoracic mammary gland on the right side of virgin mice of the C_3H, A, C57 (Black) and *dba* (Little) strains 180 days old Enlarged two times.

Fig. 4. Inset portions of third pair of thoracic glands. Silhouette of the first thoracic mammary gland on the right side of virgin mice of the C_3H, A, C57 (Black) and *dba* (Little) strains 210 to 240 days old. Enlarged two times.

Plate III

Fig. 5. Silhouette of the third thoracic mammary gland on the right side of virgin mouse of C_3H strain 165 days old. Enlarged four times.

Fig. 6. A section cut parallel to the skin surface of inset, (1), Fig. 3. ×80.

Fig. 7. Proliferation of epithelial cells lining a mammary duct from Fig. 6. ×400.

648 45 — Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore

OBSERVATIONS ON THE SEASONAL DISTRIBUTION OF THE PLANKTON OF THE TRIVANDRUM COAST*

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Received December 12, 1944

(Communicated by Dr. H. Srinivasa Rao, F.A.Sc.)

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Introduction

The importance of plankton study in fisheries research cannot be over-emphasised, though in India the subject is still in its infancy. While reviewing the progress of zoological research in India, since the inauguration of the Indian Science Congress in 1914, Rao (1938) tersely remarked, "Our

* Part of the thesis submitted for the Degree of Master of Science of the University of Travancore.

knowledge of the plankton of the Indian coasts is still very meagre, but valuable contributions have been made during the period under review by Hornell and Nayudu (1923), and Aiyar, Menon, and Menon (1936), and Menon (1931) who studied the seasonal variation of the planktonic elements along the west and east coasts of Southern India respectively". Barring the pioneer contributions by these workers on the west and east coasts, very little is known about the seasonal distribution of the plankton in Indian seas.

The present paper is the result of a continuous investigation of the plankton of the Trivandrum coast during the period, July 1938 to June 1940. The purpose of the investigation was to make a preliminary quantitative and qualitative survey of the plankton of the Trivandrum coast, and to study the seasonal variation of the principal planktonic organisms, their inter-relationships and the general factors governing their distribution. The present work conforms to the generally accepted principle that in a study of the plankton of an ocean area, previously virgin ground in this respect a general qualitative and seasonal survey is the first task (Bigelow, 1926).

Thus the investigation consisted mainly of a quantitative and qualitative examination of 273 plankton samples collected twice a week during the two years.

Physical Features and Climate of Travancore

Travancore, one of the foremost fishing areas in India, lies between latitudes 8° 4′ N., and 10° 21′ N., and longitudes 76° 13′ E., and 77° 37′ E., separated from British India by the Western Ghats on the east and bounded by the Arabian Sea on the west. Along the western boundary close to the seacoast is a chain of lakes or backwaters which extends over nearly two-thirds the length of the State from the northern frontier to the city of *Trivandrum*. These backwaters are either expansions of rivers at their mouths or extensive sheets of water receiving the flood waters of several rivers arising from the Western Ghats. They are separated from the sea by a bar of sand the width of which varies from seven miles to half a mile or less.

The climate of Travancore is equable and damp. In the major part of the State the temperature varies between 70° F. and 90° F., and hardly falls below 70° F. or rises above 90° F.

The annual rainfall is variable and its distribution seems to be determined by the distance of the locality from the northern boundary and by its distance from the hills. Travancore gets its rainfall from two monsoons; the south-west monsoon which sets in during May or June, and the north-east monsoon

which starts in October or November. The greater part of the rainfall is derived from the south-west monsoon.

Trivandrum and the Neighbouring Seas

In the vicinity of the Trivandrum coast, about four miles to the south of the Shankumughom beach, is the mouth of the Karamana river (42 miles in length) which opens into the sea through the permanent bar near Poonthurai. During the monsoon, the Veli lake, about one and a half miles to the north of the Shankumughom beach, is also in connection with the sea. The proximity of the river-mouth and the lake to the area surveyed, seems to be of considerable significance as far as the distribution of the fauna and flora of the coast is concerned.

Trivandrum has a mean annual rainfall of 67·22 inches. Rainfall is heaviest in June and July, breaking open the Veli bar and flooding the Karamana river. The coastal current is chiefly in the south-east direction during the south-west monsoon. The north-east monsoon period lasts from October to January. The direction of the coastal current then is generally to the north. Sewell (1937) has stated that with the establishment of the north-east monsoon there is a strong flow of water from the Pacific into the Indian Ocean through the straits of Sumatra. The current known as the north-east monsoon drift sweeps up the south coast of Ceylon, from thence running up the west coast of Ceylon and India. From March to May the coastal current is found to be running to south-east. However, the general trend of the current during each period shows noticeable fluctuations, and Appendix I shows the number of days during each month from July 1938 to June 1940, when the general trend of the current was reversed.

An account of the physical and meteorological conditions of the neighbouring seas is to be found in Sewell's monograph (1925–1929) on the geographic and oceanographic researches in Indian waters. He (*op. cit.*, p. 212) observed that in different parts of the Indian seas, there is a fall of temperature during the closing months of the year; in the Laccadive Sea, however, this sets in during November, reaching the minimum in January. From February it rises again till April, after which the surface temperature of the sea-water appears to fall again. According to Sewell a considerable range of variation in the surface temperature occurs, especially during those months in which temperature depends on the strength of the monsoon. Similarly, surface salinities also, which depend largely on local meteorological conditions, seem to fluctuate considerably in response to the intensity of the monsoon. However, as Sewell (*op. cit.*, p. 338) has pointed out, the north-east monsoon in this area is without any very appreciable effect on the

salinity of the surface waters, and thus on the east and west coasts of peninsular India, physical conditions of the sea are found to be entirely different during the winter months.

Methods

For elaborate and comparative work on plankton most modern and efficient methods and appliances have been devised in foreign countries. Also, tow-net hauls, both horizontal and vertical, made for accurate work, should be with standard nets, time and speed. The International Council for the Exploration of the Sea have recommended the adoption of special 'closing nets' of bolting silk (Fowler and Allen, 1928). In the present study the methods of plankton collection were, of necessity, very simple. A local fisherman, with specified instructions, was engaged to bring in the tow-net samples to the laboratory. Horizontal hauls were made from an ordinary catamaran for about ten to fifteen minutes, beyond the six fathom line with a limit seawards to about the fifteen fathom line. The usual time for making tow-net hauls was between 5 and 6 A.M. and the tow-net used was of fine muslin. The length of the coast surveyed during the investigation was about four miles between the mouth of the Karamana river in the south and the Veli lake in the north.

A cursory examination of each day's collection of plankton was done in the fresh condition, and the relative abundance of the important organisms was tabulated in an arbitrary manner by using different terms such as 'swarms', 'plenty', 'abundant', 'common', 'rare', etc. (Fish, 1925 and Menon, 1931). This made the later examination of the organisms easier and comparable.

The collection was then preserved in 4% formalin and seawater and put aside for more detailed examination and enumeration of organisms later on. To find the approximate numbers of the various planktonic elements in each collection, the preserved samples were examined as follows:

The plankton catch was poured into a large measuring jar and allowed to settle undisturbed for twenty-four hours. After noting the volume of the plankton, enough water was added to make a suitable suspension of the organisms in the fluid. The volume of water added varied according to the volume of the catch, but the proportion between the two was kept constant so that the results obtained with the different collections might be comparable. The liquid was then thoroughly stirred. As soon as the plankton organisms became evenly distributed in the fluid, four separate samples, each of 10 c.c., were taken out with a wide-mouthed measuring pipette. From each sample, larger organisms like *Sagitta*, *Leucifer*, *Salpa*, etc., were picked out separately and counted. The rest of the sample consisting mainly of the microplankton

was poured out little by little on slides and the numbers of the various organisms counted. For the enumeration of the microplankton organism the method used by Bullen (1908) was followed. The larger elements of the microplankton such as copepods, ostracods, etc., were counted under low magnifications ranging from 20 to 60 and the smaller phytoplankton under higher magnifications ranging from 60 to 200. If the first two samples did not vary by more than 10 % from their average, the remaining samples were not counted. If the difference was greater, following Clarke (1933), the third and, if necessary, the fourth sample was also counted.

In spite of all the care taken during the course of the work, the method adopted has its own limitations. In the first place, it was not easy to fix upon a common standard for measuring the comparative abundance of the various organisms in a definite volume of water. For example, a thousand specimens of *Ceratium* in, say, 10 c.c., would show a much smaller mass representation than a thousand specimens of the copepod *Temora*. In other words, the standard for measuring the abundance of the copepods could not be the same as that for the phytoplankton. It is to minimise discrepancies of this kind that different magnifications have been used for the enumeration of the copepods and the phytoplankton. In the case of *Sagitta* and other macroplankton also, the same difficulty was encountered. But as Allen (1935) has pointed out, constant and close acquaintance with the materials proves a good practical corrective for this discrepancy.

Secondly, it was not possible to determine the speed of the catamaran, the velocity of the currents, and other hydrographical factors. Nor were the physico-chemical conditions of the sea investigated. Though the present quantitative work may fail to show the exact numerical prevalence of any particular species of the plankton, it may serve as a rough estimate of the intensity and periodicity of the principal elements of the plankton.

Seasonal Distribution of the Planktonic Elements

Diatoms

The most common and important diatoms of this coast belong to the four genera, *Coscinodiscus*, *Rhizosolenia*, *Chætoceras* and *Biddulphia*. They occur almost throughout the year and form the chief constituents of the diatom flora though their maximal periods differ slightly. Of these *Biddulphia* appear in the plankton only in smaller numbers. Other diatoms like *Asterionella*, *Nitzschia* and *Ditylium* have a distinct periodicity in occurrence during the year, whereas a few forms such as *Eucampia* do not show any marked variation in numbers. The period of occurrence of the common diatoms are given in the following table:—

Diatoms	Period of occurrence
Genus *Coscindodiscus* Ehr.	
1. *C. excentricus* Ehr.	Very common during September to March.
2. *C. concinnus* W. Smith	January to April and August to October.
3. *C. granii* Gough.	Swarms during July to November.
4. *C. lineatus* Ehr.	Appears along with the preceding form at intervals.
5. *C. radiatus* Ehr.	Not very common. Occurs sparingly throughout the year.
Genus *Planktoniella* Schutt	
6. *P. sol* Schutt	Quite common during June to October.
Genus *Biddulphia* Gray	
7. *B. sinensis* Gray	Occurs throughout the year. Has a maximum during March to April.
8. *B. mobiliensis* (Bail) Grun. ..	Common along with the preceding form; but more abundant during January to May.
9. *B. regia* Schulze	Occurs in fairly good numbers throughout the year.
10. *B. granulata* Röper	A rare form occurring in chains at intervals during July to February.
Genus *Rhizosolenia* (Ehr.) Brightwell	
11. *R. semispina* Hensen	Swarms during March to May.
12. *R. shrubsoleii* Cleve	Occurs during almost all the months of the year: has a maximum during February to March.
13. *R. setigera* Brightwell	Appears in fairly large numbers from December, attaining the maximum intensity during March to May.
14. *R. acuminata* (H. Perag) ..	Time of occurrence same as that of the previous form.
15. *R. calcaravis* Schulze	Quite common during January to May.
16. *R. robusta* Norman	Appears from September to January in good numbers, swarms during November to December.
17. *R. stolterfothii* H. Perag	Occurring in spirals from February to May; March is the month of maximum intensity.
18. *R. alata* Brightwell	Common during March to May.
Genus *Chætoceras* Ehr.	
19. *C. decipiens* Cleve	Swarms occur during March to October; maximum intensity during May to June.
20. *C. debile* Cleve	Present along with the preceding form, in huge swarms during May to July.
21. *C. boreale* Bail	Common from May to September, invariably harbouring vorticellid infusorians.
22. *C. sociale* Lauder	Present at intervals from July to September and January to May.
Genus *Bacteriastrum* Shadb.	
23. *B. varians* Lauder	Occurs in small numbers from January, attaining the maximum intensity during April to May. In June disappears and appears again during August to September along with *Chætoceras* spp.

Diatoms	Period of occurrence
Genus *Asterionella* Hassal	
24. *A. japonica* Cleve	Begins to appear from April to September; has the maximum in July when it is quite common in the weekly plankton collections.
25. *A. bleakleyi* W. Smith	A rare form occurring from January to May.
Genus *Thalassiothrix* Cleve & Grunov	
26. *T. nitzschioides* Grun.	Very common during February to October in all collections; has two maxima, one in March and the other during July to September.
27. *T. frauenfeldii* (Grun.) Cleve & Grun.	Occurs during March to July.
Genus *Climacodium* Grun.	
28. *C. frauenfeldianum* Grun.	Appears in small numbers from January to August; no fixed maximum.
Genus *Streptotheca* Schrub.	
29. *S. thamensis* Schrub.	Occurs in small numbers at intervals during January to October; shows a feeble maximum during April to May.
Genus *Nitzschia* Hassal	
30. *N. seriata* Cleve	Huge swarms during March to July; presents a noticeable maximum during May.
31. *N. closterium* (Ehr.) W. Smith ..	Occurs occasionally during July to November.
Genus *Ditylium* L. Bail	
32. *D. brightwelli* (West) Grun. ..	Great swarms occur during March to May often forming the bulk of the diatom flora : not common during the rest of the year.
Genus *Stepanopyxis* Ehr.	
33. *S. turris* (Grev.) (?)	Very common from January to May; occurs in small numbers during August to September; in 1939, May was the period of maximum abundance and in 1940, March.
Genus *Fragilaria* Lyngb.	
34. *Fragilaria* sp.	Present in large numbers from June to October; rare at other times of the year.
Genus *Eucampia* Ehr.	
35. *E. zodiacus* Ehr.	Occurs in small numbers at intervals during March to September.
Genus *Detonula* Bergon	
36. *D. schroderii* Berg.	A more common diatom than the previous one, appearing in fairly large numbers during January to May; never present at other times of the year.
Genus *Thalassiosira* Cleve	
37. *Thalassiosira* sp.	Occurs in very huge swarms, arranged irregularly in mucilaginous masses, along with *Pleurosigma* sp.; in May and June 1940, it appeared in such great swarms that the water was choked as it were with *Thalassiosira*.

Diatoms	Period of occurrence
Genus *Pleurosigma* W. Smith	
38. *Pleurosigma* sp.	Present in all collections from May to November ; rare during other months ; swarms occurred during May and June 1940, to the exclusion of the common diatoms.
Genus *Corethron* Castr.	
39. *C. criophilium* Castr.	A rare diatom appearing during September to January.
Genus *Lauderia* Cleve	
40. *L. punctata* Karsten (?)	Appears from January to September at intervals.
Genus *Bellerochea* V. Heurck	
41. *B. malleus* (Brightwell)	Occurs in small numbers during January to May; in May it is quite common.
Schizophyceæ	
Genus *Trichodesmium* Ehr.	
42. *T. erythræum* Ehr.	Occurs in huge swarms during December to May at intervals ; in April 1940 appeared abundantly in all weekly collections to the exclusion of some of the common diatoms.
43. *T. theibauti* Gomont	Often present along with the preceding form, but only in small numbers.

Protozoa

No real estimate of the fecundity of these minute organisms can be made unless special methods are devised to collect them (Fish, *loc. cit.*, and Menon, *loc. cit.*). Many of the forms like Gymnodinians and Peridiniales are so minute that they escape through the meshes of the ordinary muslin net. Due to want of facilities it was not, however, possible to try Lohman's (1908) method of filtering the sea-water through hardened filter-paper and taffeta silk, or of Lebour's (1917) of taking water samples direct from the sea and centrifuging them in tubes with pointed end. But a detailed study of these organisms, especially, the 'red water' Gymnodinians is important as their occurrence in the coastal waters in unusual swarms during the monsoons, seems to have some direct influence on other plankton organisms (*vide infra*).

It is of interest to note that the seasonal distribution of the protozoans, particularly of the dinoflagellates, is very markedly related to that of the diatoms. The May maximum of the diatoms is found to be closely succeeded by the dinoflagellate maximum [see Text-fig. 1, (*b*)]. Fish (*loc. cit.*) has observed that as soon as the diatoms have exhausted their food supply and disappeared, the protozoa that utilize the nitrates and not the silicates, increase rapidly. But just why they should follow immediately after the diatoms cannot be explained though it is quite evident.

Of the protozoans, the dinoflagellates form a very important constituent of the plankton, though compared to the diatoms their number is poor in the

collection. However, during their maximal periods, they have been noticed in large quantities along with diatoms, in the stomach contents of the sardines and mackerels of this coast. Provided with locomotar flagellæ and chlorophyll, many of these dinoflagellates may be more responsive to light and darkness and this vertical distribution factor, perhaps, accounts for the dinoflagellate abundance in the gut contents of the fishes.

The seasonal occurrence of the protozoans is given below:—

Protozoans	Period of occurrence
1. *Gymnodinians*	.. Appear in huge swarms during June to September at intervals, especially after very heavy rains*: they are comparatively rare during the rest of the year.
Genus *Prorocentrum* Ehr.	
2. *P. micans* Ehr.	.. Quite common during April to September.
Genus *Diplosalis* Bergh.	
3. *D. lenticula* Bergh. ..	.. Appears in fairly large numbers during May to December.
Genus *Peridiniopsis* Lemm.	
4. *P. rotunda* Lebour ..	.. Period of abundance same as that of the previous form.
Genus *Dinophysis* Ehr.	
5. *D. homunculus* Stein ..	.. Common during September to February; appearing in rings.
6. *D. miles* Cleve	.. Occurs along with the preceding form in regular rings.
Genus *Ornithocercus* Stein	
7. *Ornithocercus* sp. ..	.. Very common during July to November in swarms.
Genus *Peridinium* Ehr.	
8. *P. depressum* Bailey ..	.. The commonest form occurring throughout the year; has a maximum during September to November.
9. *P. oceanum* Vanh. ..	.. Common from April to September; present in greatest numbers during May.
10. *P. ovatum* Pouchet ..	.. Appears at intervals from May to September.
11. *P. conicum* (Grun.) ..	.. Occurs in small numbers during August to November.
Genus *Ceratium* (Schrank)	
12. *Ceratium* sp. (?)	.. The rarest of all ceratians found on a few occasions during October and November in 1939.
13. *C. breve* Ostf. & Schmidt ..	.. Not very common; no regular time of appearance, often occurring along with other species.
14. *C. schmidti* Jorg. ..	.. Present occasionally in the plankton in very small numbers during October to November.

* When Gymnodinians occur in unusual swarms just after heavy rains, a putrid smell is often emitted from the coastal waters. On such occasions, samples of sea-water were collected and allowed to settle for over twenty-four hours, and the thin sediment formed in the vessels on examination was found to contain myriads of these Gymnodinians.

Protozoans	Period of occurrence
15. *C. fusus* Ehr.	Occurs in fairly good number during September to November at intervals.
16. *C. furca* Ehr.	Often appearing along with the previous form in smaller numbers during September to October.
17. *C. trichoceras* Kofoid (?)	Appears in good numbers throughout the year; more common during April and May.
18. *C. massiliense* Gourret	Occurring all through the year, and showing a maximum during April to May.
19. *C. tripos* O. F. Muller .. .. var. *subsala* (Ostf.)	Common throughout the year; has a maximum during September to October.
var. *lineata* (Ehr.)	Do.
Genus *Noctiluca* Lamarck	
20. *N. miliaris* (Macartney)	Most common during July to October.
Ciliated Infusorians	
21. *Tintinnus* sp.	Common from November to April; decreases in number with the onset of the monsoon in May.
Radiolarians	
Genus *Acanthometron* Popofsky	
22. *A. pellucidium* J. Muller	Quite common during October to March.

Cœlenterata

Hydromedusæ.—Hydromedusæ form the dominant group of the macroplankton from November to April. They occur in greatest numbers during January to February. During May and June they become rare. But in July, with the onset of the monsoon, swarms of Hydromedusans like *Liriope, Olindias* and *Zygocanna* and Siphonophorans like *Physalia, Porpita* and *Diphyes* appear. The commonest Leptomedusæ are *Phortis, Obelia* and *Aequorea*; the Trachomesusæ, *Liriope,* and the Narcomedusa, *Solmundella.* Most of these Hydromedusæ seem to have double periodical appearance or abundance. The first appearance of abundance during the early part of the year, January to April, is the largest and lasting for a longer time. The second periodical abundance of these common forms, caused mainly by currents, is rather erratic and occurs at intervals between July and December.

The following table shows the period of occurrence of the common Hydromedusæ of this coast:—

Hydromedusæ	Period of occurrence
Leptomedusæ	
1. *Obelia* sp.	Occurs in good numbers during January to March: present at other times of the year also.
2. *Phortis* sp.	Common throughout the year; swarms occur during November to March and July to August.
3. *Eutima* sp.	A rare form appearing along with *Phortis* during May to July.

	Hydromedusæ	Period of occurrence
4.	*Aequorea* sp.	Appears in good numbers during January to March.
5.	*Zygocanna* sp.	Common during Fedruary ; swarms in July.
6.	*Olindias singularis* Browne ..	Occurs from June to September.
7.	*Liriope tetraphylla* (Chamisso & Eysenhardt)	One of the commonest Hydromedusæ appearing in great numbers during February to March and July to August.
	Narcomedusæ	
8	*Solmundella bitentacula* Browne ..	Very common during December to March ; appears in good numbers during July also.
	Siphonophora	
9.	*Physalia* sp.	Appears occasionally during July to August.
10.	*Porpita* sp.	Often appears along with *Physalia* during July
11.	*Diphyes* sp.	Swarms are usually present in the plankton rom January to April at intervals : in July also swarms occur.
	Ctenophora	
12.	*Pleurobrachia* sp.	Very common during December to April.
13.	*Beroe* sp.	Occurs in large numbers during January to March at intervals.

Polychætes

Polychætes form an important and common constituent of the plankton. The majority of these are larval and post-larval forms of Eunicids, Nereids, Spionids, Mitrarids, Terebellids, etc., and occur almost throughout the year. The commonest pelagic adult worm is *Tomopteris*, appearing during February to April. Polychætes are most abundant during March to August. Swarms of post-larval polychætes usually occur during the heavy rains of the monsoon period. On stormy days adult *Nereis* and Turbellarians have also been found in the plankton.

Chætognatha

Sagitta presents a uniform distribution from December to April. During these months they appear regularly in all weekly collections. With the onset of the south-west monsoon, their occurrence is somewhat erratic. Huge swarms often appear at intervals from May to September. *Sagitta* is very rare during October and November (See Text-Fig. 1, *c*).

John (1937) found that the surface distribution of the *Sagitta* of the Madras coast was mainly governed by the local rainfall and that the changes of salinity brought about by the admixture of rain-water in the superficial layers of the sea might be a reason for the scarcity of *Sagitta* during the rainy season. On this coast, however, swarms of *Sagitta* have been recorded during the monsoon months when the salinity of the coastal waters is naturally

lower than that during the rest of the year. It, therefore, seems probable that the intensity of *Sagitta* during the rainy season is not so much to be attributed to changes of salinity as to other factors such as, available light, currents, etc. It is a well-known fact that during the monsoon period from June to September we have days of intermittent sunshine, and on such days many of the zooplankton elements like *Sagitta* may be stimulated to move vertically downwards and upwards to adjust themselves to their respective light optima. After the monsoons, from December to April, when the light intensity is more or less constant and uniform, the up and down vertical migrations of *Sagitta* will be less marked, and their number at the surface of the sea more uniform. Russell (1931 and 1933) and Gardiner (1934) observed that differences between the day and night catches of *Sagitta* were due to a proportion of the population moving downwards to the levels (which were influenced by light) below those fished by the nets. Thus it seems that the intensity of *Sagitta* during the monsoon months is, to a considerable degree, influenced by the vertical distribution factor, light. The stormy weather conditions prevailing during that period may also as suggested by Hardy and Gunther (1936), affect the vertical distribution of these comparatively sluggish animals so as to account for their swarming occurrences. In fact, we know very little of the vertical distribution of *Sagitta* in Indian waters and so, it will be hazardous, as observed by John (*loc. cit.*), to dilate on this subject any further without sufficient data.

Chætognatha is represented by the genera *Sagitta* and *Spadella*. The commonest species of *Sagitta* occurring on this coast are *S. enflata* and *S. regularis*; and the period of occurrence of the different species of the phylum is given below:—

Chætognatha	Period of occurrence
Genus *Spadella* Langerhans	
1. *S. cephaloptera* Hertwig	Recorded once on 15th July 1939 along with swarms of *Sagitta enflata*.
Genus *Sagitta* Slabber	
2. *S. enflata* Grassi	Swarms occur during July to September; very rare during October to November.
3. *S. regularis* Aida	Abundant during March to April and July to August; rare at other times of the year.
4. *S. tenuis* Conant	Common during July to September.

Copepods

As in other places in the Indian coast and elsewhere copepods form the chief, true pelagic crustacea of the local plankton. They are always present in great abundance except during the diatom maximum and a distinct perio-

dicity in their occurrence is noted every year. The copepods common to this region may conveniently be grouped into the following three classes:—

(1) annually appearing species which often form the bulk of the copepod fauna,

(2) annually appearing species which do not occur in such large swarms and

(3) regular visitants showing no sharp maximum and are present in the plankton only for a short time.

Class (1) is represented mainly by the genera *Oithona, Acartia, Pseudodiaptomus, Temora, Acrocalanus* and *Paracalanus*. All these copepods, excepting *Temora*, seem to be typically neritic and show the maximum intensity during December to March. The *Oithona* maximum is during January to March and the *Acartia* and *Pseudodiaptomus* maxima during December to February. With the beginning of the diatom intensity in March these copepods dwindle considerably in number, and are very rare in May and June. From October onwards their number gradually increases till their respective maximal periods.

Paracalanus and *Acrocalanus* show a slightly different form of distribution in that they do not present the sharp and abrupt maximum of *Oithona, Acartia* and *Pseudodiaptomus*. In other words, they occur in considerable numbers all through the year. *Paracalanus* appears in greatest numbers during October to December and *Acrocalanus* during December to March.

Temora shows a distribution which seems characteristic of the species. They are very rare from December to February when the common neritic species have their maxima. From April *Temora* species increase in number and in May they form a dominant part of the copepod fauna. From June to August there is a marked diminution in their number, but from September onwards *Temora* appears in swarms and this maximum lasts till November.

Class (2) includes the species of *Euterpina, Corycæus, Oncæa, Eucalanus* and *Centropages*. Of these the first two genera are quite common throughout the year. *Euterpina* has the largest maximum during July and *Corycæus* in May. *Oncæa* species appear in largest numbers in December and occur sparingly at other times of the year. *Eucalanus* and *Centropages* have the greatest maximum during April to May when they are found to occur along with *Temora, Corycæus* and *Euterpina*.

Class (3) consists of the regular visitants like *Rhincalanus, Labidocera, Candacia, Tortanus, Calanopia, Sapphirina, Miracia, Lucicutia, Euchæta, Clytemnestra, Copilia*, etc. They have never been noted to occur in great

swarms, though a few of them, for instance, *Rhincalanus* and *Tortanus* appeared on one or two occasions in fairly large numbers. Forms like *Miracia*, *Euchæta* and *Copilia* were rare throughout.

The period of occurrence of the common species of copepods is shown in the following table:—

Copepods	Period of Occurrence
Genus *Canthocalanus* A. Scott	
1. *C. pauper* (Giesbrecht)	A rare form appearing during December to February.
Genus *Undinula* A. Scott	
2. *U. vulgaris* (Dana)	Common during December to August in fairly good numbers.
Genus *Eucalanus* Dana	
3. *E. subcrassus* Giesbrecht	The commonest *Eucalanus* of this coast occurring throughout the year; rare during August to September, but appears in large numbers during April to June and November to December.
4. *E. crassus* Giesbrecht	Occurring along with the previous species during March to September.
5. *E. attenuatus* (Dana)	Present at intervals during April to October.
Genus *Rhincalanus* Dana	
6. *R. cornutus* Dana	Occurs during May to August; rare at other times of the year.
Genus *Paracalanus* Boeck	
7. *P. parvus* Giesbrecht	Present throughout the year: has a maximum during October to December.
Genus *Acrocalanus* Giesbrecht	
8. *A. longicornis* Giesbrecht	The commonest species of the genus appearing in constant numbers during November to March. Present during other months also.
9. *A. monachus* Giesbrecht	Occurring along with the preceding form.
10. *A. gracilis* Giesbrecht	Period of occurrence the same as that of the preceding forms.
Genus *Calocalanus* Giesbrecht	
11. *Calocalanus* sp.	Occurs in scanty numbers during July.
Genus *Euchæta* Phillipi	
12. *E. marina* (Prestandrea)	Occurred sparingly in December 1938 and March, 1940.
Genus *Centropages* Kroyer	
13. *C. furcatus* (Dana)	Very common during November to May; has the maximum during March to May.
14. *C. tenuiremis* Thompson & Scott ..	Occurs during March to July; absent during the rest of the year.
Genus *Pseudodiaptomus* Herrick	
15. *P. annandalei* Sewell	A very common species constituting the bulk of the zooplankton during December to February; from March onwards decreases in number till June, appearing again in swarms from August.

Copepods	Period of occurrence
Genus *Temora* W. Baird	
16. *T. discaudata* Giesbrecht	Occur in fairly good numbers during March to September at intervals.
17. *T. turbinata* (Dana)	Rare during December to February, but from March appears in good numbers and swarms are found in May ; becomes rare during June to August, only to appear again in great swarms during September to November.
Genus *Lucicutia* Giesbrecht	
18. *Lucicutia* sp.	Occurs sparingly during June to September.
Genus *Candacia* Dana	
19. *C. truncata* (Dana)	Common during March to December at intervals.
20. *C. bradyi* A. Scott (?)	Often appearing along with the previous form.
Genus *Calanopia* Dana	
21. *C. elliptica* (Dana)	Occurs in small numbers during December to May at intervals.
Genus *Labidocera* Lubbock	
22. *L. acuta* (Dana)	Present from November to April in fairly good numbers.
Genus *Pontella* Dana	
23. *P. danæ* Giesbrecht	Appears along with the preceding species.
Genus *Acartia* Dana	
24. *A. erythrea* Giesbrecht	A very common copepod appearing during all the months of year ; December to March is the period of maximum abundance.
Genus *Tortanus* Giesbrecht	
25. *T. barbatus* (Brady)	A common form occurring in fairly good numbers during March to May ; present sparingly during December to February also.
Genus *Oithona* Baird	
26. *O. rigida* Giesbrecht	One of the commonest species appearing in all weekly collections ; has a large maximum during January to March when it appears along with *Acartia* and *Pseudodiaptomus* species.
27. *O. plumifera* Baird	Quite common at intervals during March to September ; in July and August 1938 occurred in huge swarms along with the cladoceran, *Podon*.
Genus *Longipedia* Claus	
28. *Longipedia* sp.	Appears sparingly during November to May.
Genus *Euterpina* Norman	
29. *E. acutifrons* (Dana)	Quite common throughout the year ; has a noticeable maximum during May to August.
Genus *Macrosetella* A. Scott	
30. *M. gracilis* (Dana)	Does not present any sharp maximum ; occurs in fairly good numbers along with the previous form during December to April.

Copepods	Period of Occurrence
Genus *Miracia* Dana	
31. *Miracia* sp.	Occurred in scanty numbers during February 1939 and 1940; absent during other months of the year.
Genus *Clytemnestra* Dana	
32. *C. rostrata* (Brady)	Appears in small numbers during July to December.
Genus *Oncæa* Philippi	
33. *O. conifera* Giesbrecht	Common during July to December; rare during January to March.
34. *O. venusta* Philippi	Occurs along with the previous species.
Genus *Corycæus* Dana	
35. *C. obtusus* Dana	Appears in fairly good numbers during November to May; rare during June to July.
36. *C. furcifer* Claus	Common during March to July.
37. *C. venustus* Dana	Appears along with the previous form during April to May.
Genus *Sapphirina* J. W. Thompson	
38. *S. nigromaculata* Claus	Occurs in small numbers during December to May.
39. *S. ovatolanceolata* Dana	Common during December to May.
Genus *Copilia* Dana	
40. *Copilia* sp.	Occurs in scanty numbers during April to June.

Cladocera

Cladocera is represented in the plankton by *Evadne* and *Podon*. Like copepods they form important planktonic constituents. Both the genera show a more or less similar seasonal distribution. They are very rare during December to March when copepods form the chief elements of the plankton. From April onwards there is an increase in their number and the period of maximum intensity is during July to October. Of the two cladocerans, *Evadne* seems to be less affected by the diatom intensity during April to May, for swarms of this species occur even during May along with various diatoms. Also, it presents a more extended period of occurrence than *Podon*.

Evadne sp.—Occurs in swarms during April to November.

Podon sp.—Appears in fairly good numbers during May to September at intervals.

Ostracoda

Although small numbers of ostracods do appear in many of the plankton collections, many of them do not seem to be true pelagic forms. However they were present in swarms during December 1938 in all weekly collections when *Salpa* formed the main constituent of the plankton. December to March may be roughly stated as the period of occurrence of the ostracods.

Amphipoda

Like the ostracods, amphipods too are not to be considered as typically planktonic in habit. Amphipods occur in the plankton occasionally during July to January. They often appear erratically during the monsoon period just after heavy rains.

Schizopoda

Schizopods are an important group among the macro-plankton elements. They are very rare during August to September though quite common throughout the rest of the year. They appear in maximum numbers during February to April.

Cumacea

Cumaceans appear in the plankton in small numbers during October to December and April to May. They have a place in the plankton similar to the preceding groups, ostracoda and amphipoda.

Sergistidæ

1. *Leucifer* sp.—Very common throughout the year; occurs in large swarms during January to May.

2. *Acetes erythræus* Nobili.—As common as the previous form; occurring in great swarms during December to April.

Larval Crustaceans

Larval crustaceans are a constant feature of the plankton throughout the year. Many of the common forms of crustaceans seem to breed all through the year while others are periodic exhibiting a regular succession of species. In the former category are the macroplankton elements such as *Leucifer, Acetes* and mysids while the copepods seem to belong to the latter.

Copepod nauplii and decapod larvæ form the bulk of the larval crustaceans throughout the year.

I. *Copepod nauplii.*—Very common throughout the year; huge swarms occur in February to March and July to October.

II. *Decapod larvæ.*—Occur during all the months of the year.

(*a*) *Zœæ.*—Common during November to April.

(*b*) *Megalopa.*—January to May.

(*c*) *Mysis* stages of schizopods and sergestids occur in plenty throughout the year; abundant during July to November.

(*d*) *Phyllosoma.*—Appears at intervals during November to March in small numbers; swarms occurred in January 1939.

(*e*) *Stomatopod larvæ.*—Alima of *Squilla* appears in swarms during November to March at intervals, often caught along with Phyllosoma larvæ.

III. *Cirripede larvæ.*—Both the nauplii and the cypris stages occur in fairly good numbers all through the year; most common during September to November and April to June.

Pycnogonida (No-body Crabs)

They occurred in scanty numbers during July in both the years. Gravely (1941) has recorded the Pycnogonida of the Madras coast as *Anoplodactylus investigatoris.* The common Pycnogonid of this coast also seems to belong to the same species, as may be gathered from Gravely's description and figure.

Mollusca

Pelagic pteropods and larvæ of the lamellibranchiata and gastropoda form the chief constituents of the planktonic mollusca.

1. *Pteropods.—Creseis* is the common form, appearing in swarms during November to April at intervals.

2. *Larvæ of Lamellibranchiata.*—present throughout the year; swarms occur in February to April and July to September.

3. *Gastropod larvæ.*—Common during December to April.

Brachiopoda

Brachiopod larvæ are common during March to May. Larvæ of *Lingula* sp. occurred in large numbers on 27th and 28th April 1939.

Echinodermata

Post-larval and larval forms of the star fishes and brittle stars are the commonest representatives of this phylum. Bipinnaria and Auricularia larvæ are comparatively very rare in the plankton. Echinopluteus and Ophiopluteus are quite common forms during September to November and March to May.

Tunicata

The Tunicates are to a large extent erratic visitants to this coast. The common forms are *Salpa, Doliolum* and *Oikopleura.*

1. *Salpa* sp.—Great swarms occur in December; during both the years appeared in the first week of the month. In January becomes very rare, but from February appears again at intervals till April thereafter totally disappearing from the plankton.

2. *Doliolum* sp.—Appears during May to August at intervals; was present in small numbers in all the weekly collections during August 1939. Rare at other times of the year.

3. *Oikopleura* sp.—Most abundant during June to August, while it appears at indefinite intervals during the rest of the year.

Cephalochordata

1. *Branchiostoma pelagicum* Gunther (?).—Pelagic forms of *Branchiostoma* are quite common during December to April. In April 1939 all the weekly collections contained *Branchiostoma* species along with a good number of their post-larvæ.

Fish Eggs and Larvæ

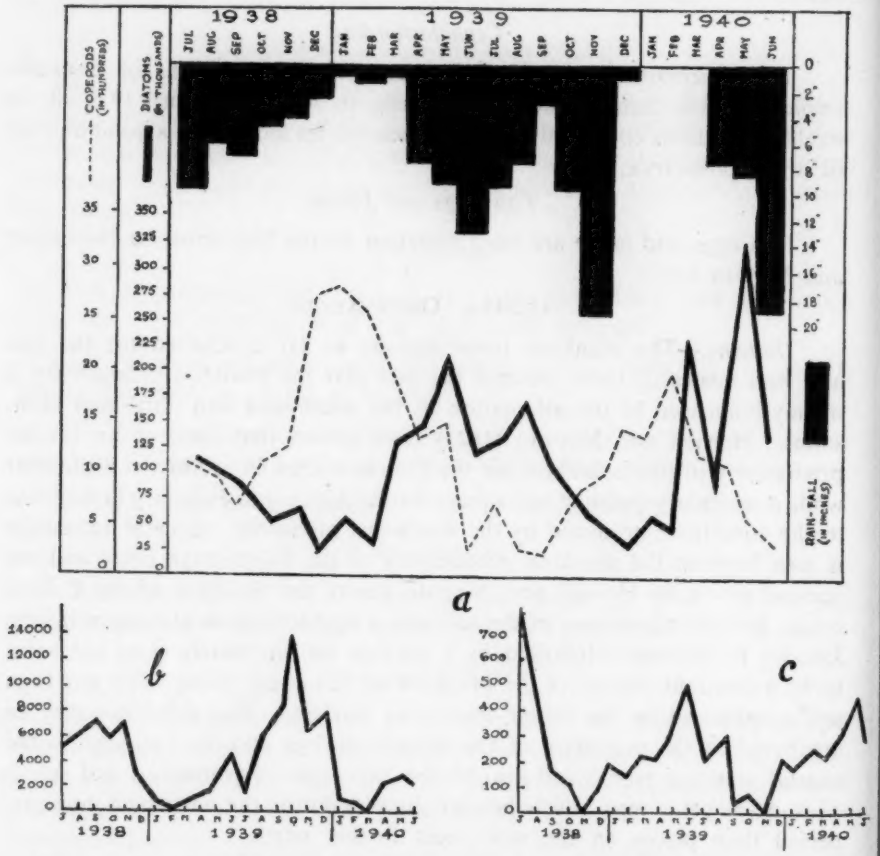
Fish eggs and larvæ are most common during September to November and May to July.

General Observations

Diatoms.—The plankton investigations so far conducted on the east and west coasts of India indicate the fact that the plankton productivity is mainly influenced by the alternation of the south-west and north-east monsoons. Hornell and Nayudu (1923) have shown that there is an intense productivity of phytoplankton off the Calicut waters from May to September while a secondary outburst takes place during January to February in response to the conditions produced by the north-east monsoon. General agreement is seen between the plankton productivity of the Trivandrum coast and the account given by Hornell and Nayudu about the plankton of the Calicut coast. But the occurrence of the secondary phytoplankton maximum during January to February, followed by a distinct fall in March, does not seem to be a constant feature of the plankton of this coast, though this has been well emphasised by the earlier west coast workers. This difference may be attributed to the proximity of the Karamana river and the Veli lake to the coastal area surveyed, and also to the fact that Trivandrum and south Travancore get comparatively heavier showers during the north-east monsoon period than places on the west coast further north.

Diatoms exhibited regular seasonal variations which were more or less similar for the two years, July 1938 to June 1940. During both the years, after the diatom scarcity during November to December, there was a gradual increase of the diatoms from January which led to the usual May maximum; and the so-called secondary maximum of the diatoms was noticed to merge with the general maximum. Again, "if we define the maximum of such a large group as the diatoms as the period when the greatest

number of forms occur in the largest number", January to February, is not in fact a period of diatom intensity, the number of species and their abundance during the months being negligibly poor when compared to the great May maximum. Thus during January to February in both the years, the main constituents of the diatoms belonged to the three genera, *Coscinodiscus*,



TEXT-FIG. 1. Chart showing (*a*) rainfall and relative abundance of Copepods and Diatoms; (*b*) seasonal distribution of Dinoflagellates; and (*c*) seasonal distribution of *Sagitta*.

Rhizosolenia and *Chætoceras*. In February 1939 and 1940, however, *Trichodesmium*, a blue-green alga also appeared in enormous numbers. But from March onwards during both the years, a sudden increase in the abundance and variety of the diatoms was observed, the predominant forms being of the genera, *Ditylium*, *Thalassiothrix*, *Stepanopyxis*, *Nitzschia*, *Biddulphia*

and *Fragilaria*. In April 1940, however, the usual swarming of these dominant species did not occur, the reason, perhaps, being the unusual appearance of large parches of *Trichodesmium* in the coastal waters, which might have caused conditions unfavourable for diatom activity of any appreciable kind. In June 1940, again, the coastal waters choked as they were with small species of *Thalassiosira* and *Pleurosigma*, had the other common diatoms in comparative rarity in the plankton collections. The diatom intensity was seen to last till the end of August during both the years, and to decrease thereafter till December (see Text-Fig. 1, *a*; and Appendix II).

A comparison of the Madras plankton with the plankton of this coast shows some remarkable differences in the distribution of many of the planktonic elements, especially, the diatoms and the dinoflagellates. While on this coast the plankton shows a clear diatom scarcity in December, which begins from the middle of September, on the Madras coast, October to December is a period of gradual diatom increase which leads to a secondary maximum during November to January. Also, a regular succession in definite order of the maxima of the three main constituents of the plankton, namely, the diatoms, dinoflagellates and copepods, is found in the Trivandrum plankton. The diatom maximum during April to May is followed by the dinoflagellate maximum during August to November, which again is succeeded by the copepod maximum during January to February. Such a regular succession of the different maxima is observed in the European seas also (Lebour, 1917). But on the Madras coast there is no separate dinoflagellate maximum: it is found to merge with the diatom maximum during April to May (Menon, *loc. cit.*). This difference may be due to the unequal rainfall on the east and west coasts during the monsoon periods.

Hornell and Nayudu noticed a definite lowering of the salinity of seawater off Calicut coast with the onset of the rains in May, and this, they thought, accounted for the phytoplankton productivity of the west coast. This phytoplankton maximum, however, is seen to occur practically at the same time of the year in the European waters as well. This fact would seem to corroborate the conclusion arrived at by Allen and Nelson (1910) that variation in salinity may be varied to a greater extent than natural variation in the sea without much effect on the rate of reproduction of the diatoms. It is therefore, to be presumed that the occurrence of the phytoplankton maximum on the west coast with the onset of the south-west monsoon is due more to the river floods bringing in large quantities of organic and inorganic food materials into the sea than to the decrease of salinity (Menon, *loc. cit.*). Nathansohn and Brandt (1909, cited by Fish) found that in the ocean far

from land, where the physical conditions are uniform to comparatively greater depths, the vertical movements of water must hasten the growth of phytoplankton through the mixing which carries nutritive substances to the illuminated zone from deeper waters. But Atkins (1928) while explaining the yearly variations in the time for the spring growth of the diatoms in the European waters, suggested that the photosynthetic diatoms which have the most rapid growth among the plankton flora, and are therefore particularly capable of utilising favourable nutrient conditions, will be most liable to be carried downwards by such movements. Marshall and Orr (1930), Gran and Barraud (1934) are also in favour of Atkin's view. In the coastal waters, however, an altogether different state of affairs exists. Fish (*loc. cit.*) has pointed out that over the deeper parts of the neritic zone, plant life is usually limited to a very thin surface layer, which differs from the deeper layers in its lesser density and higher temperature, and that in seasons when there is great outwash from the land, the neritic diatoms often form huge swarms.

How far such factors as sunlight, salinity, temperature, etc., affect the diatoms of these tropical waters is not quite evident, though it is well known that the sudden increase of the diatoms during April in the European seas is mainly attributed to the marked increase of sunlight in spring. In the temperate regions during winter, when the days are shorter and the hours of sunshine less, the diatoms do not reproduce actively, though large quantities of food materials are available in the sea at this time (Atkins, 1930). In all tropical regions where winter is not so severe, the comparatively slight variations in the total amount of sunlight and temperature do not seem to influence the diatoms considerably, which multiply gradually and reach the maximum at the same time as that in the temperate waters (Menon, *loc. cit.*).

Zoo-phytoplankton relationship.—It is an established fact that patches of phytoplankton in the sea serve as the breeding and nursery grounds for zooplankton species and so the preponderance or scarcity of the copepods and other zooplankton organisms depends to a large extent on the seasonal variations of the diatoms (Wimpenny, 1936). This does not mean, however, that the periods of phytoplankton intensity should coincide with those of the various zoo-plankton species. On the other hand, they may vary in such a way as to denote their correlationship. This is what is seen in the case of the seasonal occurrence of the copepods of this coast. The copepods are most abundant during December to March. They are most rare during June to August, though never absent from the plankton. The phytoplankton maximum reaching its intensity during May gradually decreases till

September and by December diatoms become scarce. From December begins the copepod maximum, and they are most plentiful during January when the diatoms have just begun to increase again (see Text-Fig. 1 *a*).

Though the zooplankton organisms of this coast, generally speaking, become scarce during periods of phyto-plankton intensity, some copepods like *Eucalantus, Centropages* and *Temora*, cladocerans like *Evadne* and macroplankton forms like *Lucifer*, however, do not seem to be very much affected by it. For, these have been noted to occur in good numbers even during the months of diatom intensity (see Appendix III). It therefore, seems probable that normal phytoplankton abundance does not inhibit the distribution of the zooplankton. All the same, many forms like the copepods *Pseudodiaptomus, Oithona*, etc., which appear in large numbers all through the year are markedly scarce during May to September when phytoplankton forms the dominant constituent of the plankton. This difference in the seasonal variation of the zooplankton elements is, perhaps, to be linked with their difference in the spawning seasons, rate of reproduction and such other biological factors which considerably affect the nature of plankton distribution at large.

As Johnstone (1908) has observed, plankton distribution is dependent on hydrographic and meteorological conditions also. But according to Michael Ellis (1911), any postulate concerning the distribution or abundance of plankton that fails to recognise the influence of the organisms on this distribution is inadequate. Thus the rate of reproduction, rate of growth and food relations of the marine fauna and flora do influence the distribution of plankton. This fact, again is evidenced in the work of Herdman, Scott and Dakin (1910) who in their intensive study of the marine plankton around the south end of the Isle of Man, found that the normal sequence of events in the life-history of organisms, for instance, is a dominant factor in determining the constitution of the plankton at a particular time and place.

The influence of organisms on the variety and abundance of plankton may be illustrated from another standpoint by the conditions created in the Trivandrum coastal waters due to the occasional, but unusual appearance of swarms of Gymnodinians just after heavy rains during the monsoon period. On days when Gymnodinians occurred in unusually large swarms, a bad odour, arose from the costal water, and the plankton collections were noticeably poor, being devoid of the common forms of diatoms, copepods, other crustaceans and macroplankton organisms. In 1902 Torrey (cited by Michael Ellis) has pointed out in an article entitled, "The Unusual Occurrence of

Dinoflagellates on the Californian Coast", the retarding effects of "red water" due to *Gonyaulax* on various marine animals like crabs and fishes. Similar occurrences of dinoflagellates causing mortality to fish and other marine animals have been recorded by Hornell and Nayudu (*loc. cit.*) on the Calicut coast and by Aiyar (1936) on the Madras coast.

Also, the intermittent occurrence of large parches of the blue-green alga, *Trichodesmium* in the Trivandrum coastal waters during December to April, and swarms of *Pleurosigma* and *Thalassiosira* during May to June, is seen to have a marked inhibiting influence not only on the distribution of the zooplankton species but also on the distribution of the diatoms as a whole (see Appendix II). It seems probable that the intensive reproductive activity of many of these rapidly multiplying phytoplankton species results in a considerable deficiency in the oxygen content of the coastal waters thus adversely affecting the distribution of the various planktonic elements.

What occurs to the zooplankton species during days of unusual phytoplankton intensity may be said to occur to the phytoplankton species during days of unusual zooplankton abundance. For instance, on occasions when huge swarms of *Salpa, Oikopleura, Doliolum, Sagitta* or Hydromedusæ appeared irregularly during January to March and July to August, the common phytoplankton species were found to be considerably rare in the plankton collections. The swarming occurrences of the abovementioned macroplankton organisms may, however, be attributed mainly to currents as Russell (1935) has recorded them as indicators of water movements in the sea.

To understand the relative abundance of the zooplankton and phytoplankton it is necessary to make a study of the phosphate content of the sea water also. As Hardy and Gunther (*loc. cit.*) have observed, the relative reduction in phosphate content appears to give a better index in studying the zoo-phytoplankton relationship than the phytoplankton itself. This has, however, to be left a subject for future investigations.

Acknowledgments

I wish to express here my deep indebtedness to Dr. C. C. John, Professor of Marine Biology and Fisheries, University of Travancore, Trivandrum, for his constant help, encouragement and guidance during the course of this work. I am thankful to Dr. Baini Prashad, now Fisheries Deveolpment Adviser with the Government of India, for having given me all library and laboratory facilities during the short period of my work in the Zoological Survey of India in 1941. It is a pleasure to record here my gratefulness to

Dr. S. L. Hora, Director of Fisheries, Bengal, for his kind suggestions and appreciation of my work and to Dr. H. S. Rao, Deputy Fisheries Development Adviser with the Government of India, for his valuable help and criticisms in making this paper press-ready. My sincere thanks are also due to my friend Mr. K. Sankara Pillai, Lecturer in Mathematics, University College, Trivandrum, for assistance in dealing with the statistical data on plankton.

This work was carried out during the tenure of a scholarship kindly granted to me by the University of Travancore.

Summary

An account of the general quantitative and qualitative study of the plankton of the Trivandrum coast during the years July 1938 to June 1940 is given with intensity charts and tables.

The plankton productivity of this coast is found to be affected mainly by the monsoons when the organic and inorganic matter brought down into the coastal waters through the Karamana river on the south and the Veli bar on the north of the Trivandrum beach is found to be of considerable importance in the maintenance of plant and animal life in the coastal waters.

A regular succession in the maxima of the three main constituents of the plankton—namely, diatoms, dinoflagellates and copepods—is noticed year after year. The occurrence of a secondary diatom maximum during January to February followed by a distinct fall in March (Hornell and Nayudu, 1923), does not seem to be a characteristic feature of the plankton of this area though this fact has been well emphasised by the earlier workers on west coast plankton. This difference is to be attributed to the comparatively heavier showers during the north-east monsoon in Trivandrum and south Travancore than on the west coast further north, and also to the proximity of the Karamana river and the Veli lake which open into the coastal area surveyed.

The inhibiting influence of certain forms of plankton organisms such as the Gymnodinians which constitute the so-called 'red water', the blue-green alga, *Trichodesmium* and diatoms like *Pleurosigma* and *Thalassiosira* on plankton distribution is suggested, and the general factors governing the zoo-phytoplankton relationship are discussed in the light of the observations made.

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Appendix I

Table showing the Number of Days when the General Trend of the Coastal Current is Reversed during each Month from July 1938 *to June* 1940

Months	Jan.		Feb.		March		April		May		June		July		Aug.		Sep.		Oct.		Nov.		Dec.	
Direction of current	N.S.	S.N.	N.S.	S.N.	N.S.	S.N.	N.S.	S.N.	N.S.	S.N.	N.S.	S.N.	S.N.	S.N.	N.S.	S.N.	N.S.	S.N.	N.S.	S.N.	N.S.	S.N.	N.S.	S.N.
1938	..	..	..	.	..	..	.	.	..	..	..	14	17	20	11	16	14	18	13	23	7	13	13	18
1939	6	25	10	18	16	15	20	10	21	10	19	11	16	15	19	12	20	10	15	16	15	15	10	21
1940	12	19	11	18	12	19	14	16	18	13	20	10	..	..	..	..	..	..	..	..	..	..	..	..

APPENDIX II

Seasonal Variations of the Diatoms and other Phytoplankton Organisms

(*N.B.*—The Figures denote the Number of Organisms in 10 c.c. of Seawater)

	July	August	Sept.	October	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Asterionella ..	18,500	11,110	1,230	..	..	..	..	..	..	2,800	6,300	12,000
Bacteriastrum ..	..	270	2,400	..	5,100	..	600	..	920	5 000	2.200	..
Biddulphia ..	1,600	1,700	1,300	100	2,800	900	9,800	160	18,200	21.000	23.000	8,700
Chætoceras ..	10,700	11,160	3,080	..	8,330	..	7,000	100	12,000	23,710	25,000	11,100
Climocodium ..	..	..	..	100	..	..	1,000	..	100	120	160	100
Coscinodiscus ..	1,170	1,860	12,400	25,300	28,600	14,600	7,860	14,120	28,000	24,100	10,500	2,700
Detonula ..	..	..	..	..	..	..	..	520	1,500	3,300	1,600	..
Ditylium ..	..	200	..	..	..	..	3,300	420	14,200	25,000	12,600	100
Eucampia ..	300	..	530	..	..	..	..	..	..	..	440	..
Fragilaria ..	13,580	10,380	10,200	..	100	..	..	..	..	..	..	12,300
Lauderia ..	..	1,150	..	..	..	..	2,400	..	..	..	10,800	..
Nitzschia ..	10,000	11,700	..	..	..	..	..	..	21,100	24,200	28,400	22,000
Pleurosigma ..	14,050	18,750	16,320	12,000	20,710	1,400	3,300	800	940	320	5,800	1,800
Planktoniella ..	..	410	1,400	..	..	..	..	..	..	..	..	320
Rhizosolenia ..	11,920	13,080	12,220	1,600	6,200	8,070	22,500	19,280	20,110	26.600	14,200	6,200
Stepanopyxis ..	..	950	4,620	..	280	..	8,100	400	220	580	14,100	100
Streptotheca ..	1,300	30	1,080	650	..	..	1,800	..	980	3,200	5,000	1,800
Thalassiothrix ..	11,350	1,410	12,360	..	640	..	..	180	21,100	11,200	15.400	18,000
Thalassiosira ..	25,500	17,290	9,800	12,200	..	..	..	..	..	..	37,000	19,000
Other Diatoms ..	..	..	..	..	..	..	800	150	620	430	2,100	..
TOTAL ..	119,970	101,450	88,940	51,950	72,760	24,970	68,460	36,130	139,890	171,560	214,600	116,220
Trichodesmium ..	..	..	..	..	..	..	..	12,000	2.000	1 000	..	..
Ceratium ..	1,890	2,560	4,780	1,840	3,890	840	880	70	100	200	1,000	30
Peridinium ..	840	1,330	1,740	2,010	1,840	580	110	480	370	1,330	2,010	910
Other Peridiniales	1,610	2,200	1,090	1,570	1,910	900	..	..	650	520	990	750
TOTAL ..	4,340	6,090	7,610	5,420	7,640	2,320	990	550	1,120	2,050	4,000	1,690
Radiolarians ..	..	50	..	560	300	480	60	100	210	..	680	..

APPENDIX II.—(*Continued*)

	July	August	Sept.	October	Nov.	Dec.	Jan.	Feb.	Mnrch	April	May	June
Asterionella ..	15,000	20,300	..	..	..	..	..	..	..	..	..	..
Bacteriastrum ..	..	..	..	100	..	..	150	..	600	..	6,600	..
Biddulphia ..	10,000	16,400	300	150	300	100	920	120	10,400	100	20,000	..
Chætoceras ..	9,800	14,800	18,800	5,000	800	..	3,000	..	27,300	..	49,900	100
Climocodium ..	100	..	100	150	..	..	500	..	..	..	200	..
Coscinodiscus ..	1,200	5,000	28,400	30,000	22,000	20,000	21,000	27,200	36,000	41,000	2,000	4,000
Detonila ..	..	..	..	..	..	..	1,940	..	16,000	100	1,600	..
Ditylium ..	..	..	..	..	..	..	1,100	..	14,000	10,000	31,200	..
Eucampia ..	1,000	..	9,800	..	..	..	..	..	380	..	..	..
Frägilaria ..	23,000	28,600	17,600	150	120	..	..	..	410	..	..	..
Lauderia ..	100	..	..	..	..	..	920	..	12,700	..	18,700	..
Nitzschia ..	13,000	400	700	..	..	..	1,670	..	18,000	..	56,000	..
Pleurosigma ..	2,400	27,000	12,000	10,000	26,000	1,100	300	100	180	..	25,000	60,000
Planktoniella ..	430	1,300	2,000	900	..	..	..	..	260	..	..	..
Rhizosolenia ..	12,200	16,600	11,700	18,300	9,100	11,600	19,700	31,350	26,600	22,000	18,000	1,300
Stepanopyxis ..	..	..	1,000	900	..	..	5,300	100	28,000	14,000	150	..
Streptotheca ..	5,000	100	..	150	..	..	..	60	..	..	13,300	..
Thalassiothrix ..	25,300	29,200	23,000	9,700	100	..	2,400	..	19,600	..	10,000	810
Thalassiosira ..	20,000	14,200	..	..	..	..	..	..	..	..	30,000	60,000
Other Diatoms ..	..	300	..	..	..	..	380	..	9,600	..	5,400	1,100
TOTAL ..	138,930	174,200	130,700	75,000	58,420	33,600	59,280	58,930	220,030	83,600	336,050	127,310
Trichodesmium ..	..	..	..	..	..	100,000	..	14,000	..	120,000	9,000	100
Ceratium ..	2,630	4,340	8,750	2,500	5,060	620	1,070	120	70	130	650	700
Peridinium ..	1,160	1,230	2,020	2,410	1,090	320	180	530	100	1,090	1,640	1,440
Other Peridiniales	2,000	2,380	2,460	1,730	1,860	440	..	..	240	700	800	440
TOTAL ..	5,790	7,950	13,230	6,640	8,010	1,380	1,250	650	410	1,920	3,090	2,580
Radiolarians ..	..	..	1,230	380	400	980	200	280	..	..	..	..

APPENDIX III

Seasonal Variations of the Copepods and other Zooplankton Organisms

APPENDIX III

Seasonal Variations of the Copepods and other Zooplankton Organisms

(*N.B.*—The Figures denote the Number of Organisms in 10 c.c. of Seawater)

	July	August	Sept.	October	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Acartia ..	97	76	50	26	88	490	384	784	480	214	138	10
Acrocalanus ..	78	89	44	52	153	194	188	232	204	120	128	20
Calanopia ..	..	..	..	..	..	8	7	4	2	..	2	..
Candacia ..	2	1	1	..	..	1	..	..	..	2	2	1
Canthocalanus ..	..	..	..	..	..	7	4	..	..	6	..	..
Centropages ..	23	18	11	..	8	8	..	..	7	22	88	16
Clytemnestra ..	1	2	..	4	4	3	..	..	1	..	..	..
Copilia ..	..	..	..	..	..	..	..	..	..	..	..	..
Corycæus ..	38	40	32	8	52	74	78	33	21	51	170	8
Cyclopoida, etc. ..	16	10	2	..	..	1	..	..	5	2	3	2
Eucalanus ..	55	31	10	49	26	28	26	1	..	19	58	6
Euchæta ..	..	..	..	..	..	4	..	..	..	..	..	..
Euterpina ..	72	44	20	12	118	63	..	57	68	93	167	38
Labidocera ..	1	2	..	2	3	2	4	..	1	..	..	2
Longipedia ..	..	..	..	..	2	..	1	..	..	2	..	1
Lucicutia ..	6	3	4	2	..	..	..	..	..	..	..	2
Macrocetella ..	21	..	..	..	43	58	22	14	8	38	24	21
Miracia ..	..	..	..	..	..	..	..	4	..	..	..	..
Oithona ..	120	183	111	41	152	760	1,208	946	893	605	154	20
Oncæa ..	57	47	17	68	19	144	..	..	..	28	75	11
Paracalanus ..	41	39	18	170	57	76	33	18	15	47	125	12
Pontella ..	..	1	..	2	4	..	3	1	..	1	..	1
Pseudodiaptomus	273	196	224	207	200	840	1,050	420	186	66	..	..
Rhincalanus ..	19	..	..	..	..	2	..	..	..	..	1	..
Sapphirina ..	..	2	..	6	..	..	..	..	..	..	4	..
Temora ..	108	76	231	420	341	19	15	..	..	18	360	108
Tortanus ..	..	5	3	..	..	4	2	..	..	..	15	..
Undinula ..	6	8	..	..	..	6	4	10	6	4	..	..
TOTAL ..	1,034	873	778	1,069	1,270	2,792	3,029	2,527	1,897	1,338	1,514	279
Cladocera ..	3,400	1,600	430	1,310	200	120	180	..	..	300	150	2,300
Cirripedes ..	20	40	60	110	20	..	..	10	30	100	100	120
Ostracods ..	20	..	20	180	70	260	20	210	90	30	190	20
Amphipods ..	30	110	50	70	40	90	60	40	10	..	..	20
Mysids ..	18	10	4	4	1	20	20	31	22	24	11	3
Leucifers ..	9	6	8	5	3	10	13	28	32	30	24	..
Larval Bivalves ..	340	790	250	680	200	470	250	1,800	560	2,200	370	110
Polychætes ..	220	200	110	94	130	80	60	30	90	100	120	80

APPENDIX III.—(*Continued*)

	July	August	Sept.	October	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Acartia ..	..	..	..	18	192	240	316	470	391	480	184	18
Acrocalanus ..	118	53	30	49	147	187	54	46	213	91	52	63
Calanopia ..	..	..	..	..	..	6	..	..	..	..	..	4
Candacia ..	3	1	4	4	6	2	1	..	6	..	5	4
Canthocalanus ..	..	..	..	..	..	23	4	4	..	3	..	..
Centropages ..	..	..	4	..	12	104	20	6	84	43	102	20
Clytemnestra ..	4	..	..	..	..	4	..	..	..	..	..	..
Copilia ..	..	..	..	..	..	..	..	..	..	7	4	..
Corycæus ..	70	30	12	40	38	47	46	57	40	77	26	7
Cyclopoida, etc. ..	2	1	..	..	3	16	3	14	11	80	1	7
Eucalanus ..	37	2	..	21	84	138	5	37	46	116	76	105
Euchæta ..	2	..	..	..	..	..	..	..	..	..	..	..
Euterpina ..	220	72	118	38	12	20	32	7	21	13	5	11
Labidocera ..	..	..	..	1	8	12	7	1	21	29	14	2
Longipedia ..	..	..	..	..	2	..	..	..	..	..	2	..
Lucicutia ..	2	..	..	..	..	..	..	..	..	..	..	6
Macrosetella ..	200	140	64	90	64	18	3	4	11	51	4	..
Miracia ..	..	..	..	..	..	..	..	8	..	..	..	..
Oithona ..	36	..	126	194	68	159	410	716	308	104	20	23
Oncæa ..	39	58	11	43	21	112	18	..	33	..	8	..
Paracalanus ..	53	22	..	21	133	93	27	18	48	53	37	25
Pontella ..	..	..	..	..	12	20	10	..	34	24	18	..
Pseudodiaptomus	..	..	..	50	47	209	394	680	26	7	20	..
Rhincalanus ..	20	4	..	8	..	..	..	..	..	..	19	25
Sapphirina ..	..	..	..	..	..	28	..	8	4	20	6	..
Temora ..	23	39	76	123	54	46	7	8	47	109	44	51
Tortanus ..	..	..	..	..	..	4	5	..	10	2	16	..
Undinula ..	1	..	..	..	..	6	4	3	7	9	..	14
TOTAL ..	840	422	441	700	03	1,494	1,366	2,087	1,368	1,318	633	385
Cladocera ..	1,420	630	700	210	680	100	..	..	80	390	1,860	660
Cirripedes ..	90	80	70	30	..	50	..	..	30	70	50	60
Ostracods ..	..	10	..	..	..	60	10	150	200	..	10	..
Amphipods ..	80	112	60	50	70	100	60	..	..	..	20	80
Mysids ..	..	5	..	17	9	11	8	14	16	23	4	..
Leucifers ..	4	2	4	3	6	7	7	18	25	28	22	5
Larval Bivalves ..	200	470	600	550	620	280	440	680	1,300	720	280	240
Polychætes ..	20	90	90	40	40	60	20	10	120	100	100	80

STUDIES ON HÆMOSPORIDIA FROM INDIAN BIRDS—SERIES II

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Received September 25, 1944

(Communicated by Prof. G. P. Mazumdar, Ph.D.)

(With 1 Plate)

This paper is the continuation of the series in our studies on Hæmosporidia from Indian birds and embodies the result of our observations on some species of Hæmosporidia of the families, Hæmoproteidæ and Plasmodiidæ. The parasites described here are new to science except *Hæmoproteus danilewskyi* (Grassi and Feletti, 1890) which is being reported for the first time from this part of India.

The birds were purchased from the local dealers.* The blood of fourteen different species of birds were examined, but it happened that only four of them harboured the parasites. The table at the end of this paper shows the occurrence of the parasites in different species of birds and the number of birds infected out of the total number examined. The locality of the parasites is also noted in the table.

Family *Hæmoproteidæ* Doflein (1916)

Of the two genera *Leucocytozoon* and *Hæmoproteus* of this family, only the latter is represented here.

Genus *Hæmoproteus* Kruse (1890)

During the course of our investigation we found two birds, the Indian white-eye *Zosterops p. palpebrosa* (Temm. and Schlegel), and the Indian Magpie-Robin, *Copsychus s. saularis* (Linn.), infected with *Hæmoproteus*. The parasite of *Copsychus s. saularis* is synonymous with *Hæmoproteus danilewskyi* (Grassi and Feletti, 1890), while that of the white-eye is a new

* Some of the birds were kindly procured for us by Mr. B. Biswas, B.Sc., a post-graduate student of this department, for which we are indebted to him.

one, as it does not resemble any known species of *Hæmoproteus* so far described, we propose to call the parasite *Hæmoproteus zosteropsi* n.sp.

Observations on *Hæmoproteus zosteropsi* n.sp.

The early gemetocytes are spherical in outline (Fig. 1) with a hyaline cytoplasm and a dot-like chromatin mass representing the nucleus. In these forms no pigment could be seen. As the parasites grow bigger in size, pigment granules appear in the cytoplasm and their sexual dimorphism becomes apparent.

Mature male and female gametocytes are found in abundance in the red blood corpuscles and majority of them are typically halter-shaped, while some are broadly oval in form.

A young female gametocyte (Fig. 2), measuring $2{\cdot}2\,\mu$ in diameter, is spherical in outline with a blue stained cytoplasm and circular nucleus. The cytoplasm contains scattered pigment granules. A mature female gametocyte, however, is a typical halteridium, completely filling the infected red blood corpuscle and displacing the nucleus of the latter to the periphery (Fig. 3). The cytoplasm takes up a deep blue stain and contains scattered pigment granules which are dark brown in colour. The nucleus is spherical in outline and sub-central in position. The female gametocytes measure $13{\cdot}2\,\mu \times 5{\cdot}5\,\mu$.

The early male gametocytes (Fig. 4), $6{\cdot}6\mu \times 2\mu$, are elongated bodies with hyaline cytoplasm and a rod-like nucleus. The cytoplasm contains finely scattered pigment granules which are lighter in colour than that of the female. The mature male gametocytes are mostly halter shaped (Fig. 5) while some are broadly oval in form (Fig. 6). The cytoplasm of the gametocytes appears almost unstained in Geimsa. The pigment has the same form as in the female, but they are aggregated at both poles of the parasites. The nucleus is more or less rectangular in shape, central in position, and contains chromatin granules. The male gametocytes measure $8{\cdot}8\text{–}11\,\mu \times 3{\cdot}3\,\mu$.

Affinities

The parasite under report resembles *Hæmoproteus coraciæ* de Mello and Afonso (1935) in general appearance, but differs in structural details. The female gametocytes of *H. zosteropsi* approach those of *H. coraciæ* in shape and character of the nucleus, but differ in the homogeneous nature of the cytoplasm unlike that of *H. coraciæ*. Further the pigments in *H. zosteropsi* are in the form of fine granules scattered irregularly throughout the cytoplasm and they never show any tendency to aggregate at the poles as in *H. coraciæ*. The male gametocytes of *H. zosteropsi* on the other hand,

differ from those of *H. coraciaæ* in having a rectangular nucleus, the nucleoplasm of which is highly granular.

Diagnosis

Systematic position.—Hæmoproteus zosteropsi n.sp. (Hæmosporidia, Hæmoproteidæ).

Description.—Gametocytes halter-shaped, some broadly oval in form; the female gametocytes, measuring $13 \cdot 2\mu \times 5 \cdot 5\mu$, with a spherical nucleus and pigment scattered throughout the cytoplasm; the male gametocyte, measuring $8 \cdot 8$—$11\mu \times 3 \cdot 3\mu$, with a rectangular nucleus and pigment aggregated at both the poles.

Location.—Red blood cells.

Host.—Zosterops palpebrosa palpebrosa (Temm. and Schlegel)

Locality.—Calcutta, Bengal.

Date.—June, 1944.

OBSERVATIONS ON *H. danilewskyi* (GRASSI AND FELETTI, 1890)

Plimmer (1912) reported the occurrence of this parasite in a long list of Indian birds, including the present host, kept in the Zoological Gardens, London. Recently de Mello (1937) recorded this parasite in Scops owl, *Otus bakkamæna* Pennant, from Ceylon. We have also encountered *H. danilewskyi* in the blood of the Indian Magpie-Robin *Copsychus s. saularis* (Linn.).

FAMILY *Plasmodiidæ* MESNIL (1903)

Of the three genera of the above family, *Proteosoma, Laverania* and *Plasmodium*, only the last genus is represented here.

GENUS *Plasmodium* MARCHIAFAVA AND CELLI (1885)

During our routine blood examination of birds, we found, the weaver bird *Ploceus philippinus* (Linn.) and the small minivet *Pericrocotus cinnamomeus iredalei* Baker, infected with two distinct species of *Plasmodium* respectively. As these parasites do not resemble any known species of *Plasmodium*, we propose to call them *Plasmodium ploceii* n.sp. and *Plasmodium pericrocoti* n.sp. after the names of their hosts.

OBSERVATIONS ON *Plasmodium ploceii* N.SP.

The trophozoites (Fig. 7) are irregular in outline, measuring about $2 \cdot 2\mu$ in diameter and are devoid of pigment. The cytoplasm is clear and contains a centrally placed chromatin dot representing the nucleus.

The schizonts (Fig. 8) are also irregular in outline, contain blue stained cytoplasm and a distinct mass of pigment granules. In a mature form (Fig. 9) measuring $6 \cdot 6 \mu$ in diameter, eight nuclei can be seen.

An early gametocyte is spherical in outline (Fig. 10) and measures $4 \cdot 4 \mu$ in diameter. The cytoplasm stains blue and contains a spherical nucleus. A group of fine pigment granules can also be seen.

The female gametocytes are variable in form. They may be spherical (Fig. 11) or oval (Fig. 12) in outline. The former measures $5 \cdot 5 \mu$ in diameter, while the latter $4 \cdot 4 \mu \times 3 \cdot 3 \mu$. The cytoplasm takes up deep blue stain and contains irregularly scattered pigment granules which are dark brown in colour. The nucleus is more or less sub-centrally placed and may be spherical (Fig. 11) or bi-lobed (Fig. 12) in shape.

The male gametocytes (Fig. 13) are more or less oval in form with one end broader than the other and measure $6 \cdot 6 \mu \times 3 \cdot 3 \mu$. The cytoplasm is faintly stained and contains pigment granules aggregated at one pole of the parasite. The nucleus is spindle-shaped with the ends pointed and stains lighter than that of the female.

Affinities

The present parasite approaches *Plasmodium præcox* var. *muniæ* Dasgupta and Siddons (1941). The schizonts and female gametocytes of *P. ploceii* resemble those of *P. præcox* var. *muniæ* in the number of nuclei and shape respectively; but the two parasites differ from each other in the shape and in the detailed structure of the male gametocytes.

Diagnosis

Systematic position.—Plasmodium ploceii n.sp. (Hæmosporidia, Plasmodiidæ).

Description.—Trophozoites irregular and non-pigmented; schizonts, measuring $6 \cdot 6 \mu$ in diameter, with eight nuclei; female gametocytes spherical ($5 \cdot 5 \mu$ in diameter) or oval ($4 \cdot 4 \mu \times 3 \cdot 3 \mu$), with scattered pigment granules and spherical or bi-lobed nucleus; male gametocytes, measuring $6 \cdot 6 \mu \times 3 \cdot 3 \mu$ more or less oval with one end broader than the other, nucleus spindle-shaped and cytoplasm with the pigment aggregated at one pole; infected red blood cells hypertrophied with nucleus displaced to one end.

Location.—Red blood corpuscles.

Host.—Ploceus philippinus (Linn.)

Locality.—Calcutta, Bengal.

Date.—June, 1944.

OBSERVATIONS ON *Plasmodium pericrocoti* N.SP.

The trophozoites (Fig. 14), measuring 2·2 μ in diameter, are spherical in outline with hyaline cytoplasm and a chromatin dot representing the nucleus. In these forms no pigment could be seen. Multiple infection of the red blood cells by the early trophozoites are not uncommon. As many as five parasites have been found to infect a corpuscle (Fig. 15).

The early schizonts (Figs. 16 and 17) observed by us are spherical or crescent-shaped with faint blue stained cytoplasm and two nuclei. In these forms pigment granules could not be observed. In later stage, however, (Fig. 18) pigment granules appear for the first time in the cytoplasm. A mature schizont (Fig. 19) measuring 5·5 μ in diameter is spherical in outline, with blue stained cytoplasm and pigment granules concentrated at the centre in a small dense mass. In a mature schizont sixteen nuclei can be counted. The infected red cells become distorted with the nucleus displaced to one side.

A female gametocyte (Fig. 20) measuring 9·9 $\mu \times 4·4 \mu$ is more or less oval in outline and completely fills the cytoplasm of the infected red blood cell. The cytoplasm stains deep blue and contains scattered pigment granules. The nucleus is spherical in outline and placed near one pole of the parasite.

The male gametocyte (Fig. 21) measuring 9·6 $\mu \times 4·4 \mu$ is bean-shaped with faint blue stained cytoplasm and the pigment granules aggregated at one pole. The nucleus is spindle-shaped and situated at the convex border of the parasite; it stains lighter than that of the female and contains scattered chromatin granules.

Affinities

Of the known species of *Plasmodium* from birds, the parasite under report resembles *P. præcox* (Grassi and Feletti, 1890; *vide* Wenyon, 1926). The schizonts of *P. pericrocoti* approach those of *P. præcox* in shape, number of nuclei, and in the similar clumping of pigment into a single dark mass. But the two species differ from each other in the shape, size and structure of the gametocytes.

Diagnosis

Systematic position.—Plasmodium pericrocoti n.sp. (Hæmosporidia, Plasmodidæ)

Description.—Trophozoites spherical and non-pigmented; schizonts measuring 5·5 μ in diameter with sixteen nuclei and the pigment collected

into single dense clump; female gametocytes, measuring $9{\cdot}9\mu \times 4{\cdot}4\mu$, more or less oval with scattered pigment granules and a spherical nucleus; male gametocytes, measuring $9{\cdot}6\mu \times 4{\cdot}4\mu$, bean-shaped with pigment aggregated at one pole and the nucleus situated at the convex border.

Location.—Red blood cells.

Host.—Pericrocotus cinnamomeus iredalei Baker.

Locality.—Calcutta, Bengal.

Date.—June, 1944.

TABLE

Host	Number examined	Number infected	Parasite	Locality
Acridotheres ginginianus (Lath.) ..	4	..	..	Calcutta
Aegithina tiphia tiphia (Linn.) ..	1	..	..	,,
Aethiopsar fuscus Wagler ..	2	..	..	,,
Amandava amandava (Linn.) ..	1	..	..	,,
Copsychus saularis saularis (Linn.) ..	2	1	*Hæmoproteus danilewskyi* (Grassi and Feletti)	,,
Leptocoma zeylonica (Linn.) ..	1	..	..	,,
Munia artricapilla rubronigra (Hodgs.)	1	..	..	,,
Munia malacca malacca (Linn.) ..	3	..	..	,,
Pericrocotus cinnamomeus iredalei (Baker)	2	2	*Plasmodium pericrocoti* n.sp.	,,
Ploceus philippinus (Linn.) ..	2	2	*Plasmodium ploceii* n.sp.	,,
Sturnopastor contra contra (Linn.) ..	1	..	..	,,
Uroloncha malabarica (Linn.) ..	1	..	..	,,
Uroloncha punctulata punctulata (Linn.)	1	..	..	,,
Zosterops palpebrosa palpebrosa (Temm. and Schlegel)	4	2	*Hæmoproteus zosteropsi* n.sp.	,,

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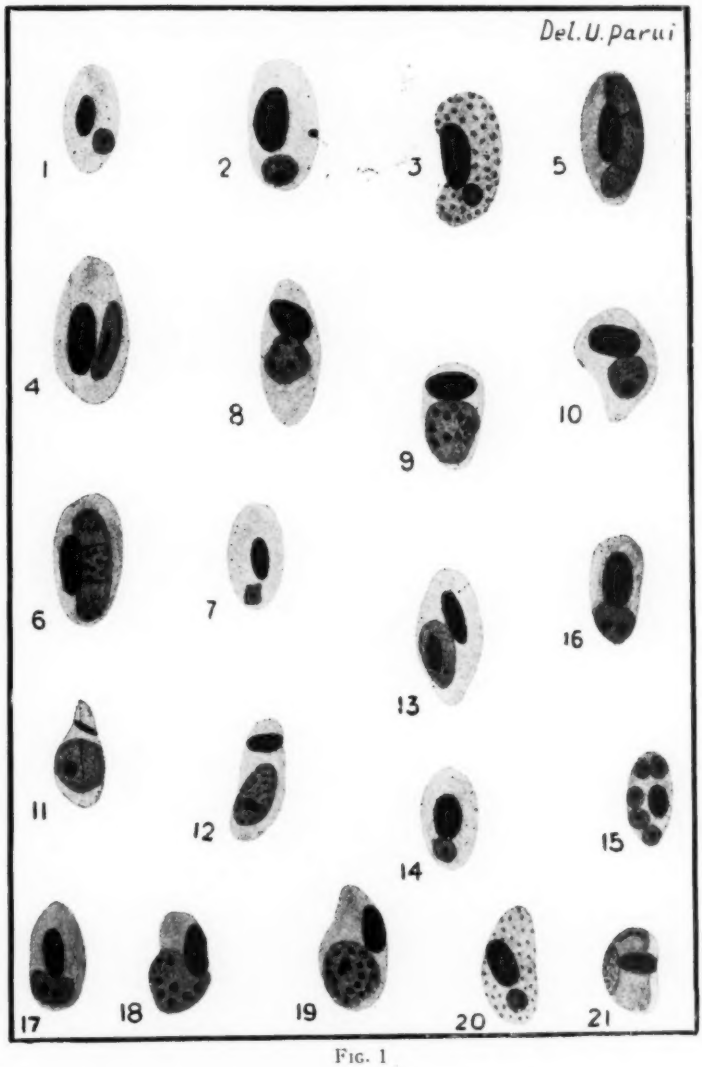

FIG. 1

Explanation of Plate IV

All figures are magnified 1,660 times

Figs. 1–6. *Hæmoproteus zosteropsi* n.sp.

Fig. 1. An early gametocyte.
Fig. 2. A young female gametocyte.
Fig. 3. A mature female gametocyte.
Fig. 4. A young male gametocyte.
Figs. 5 & 6. Mature male gametocytes.

Figs. 7–13. *Plasmodium ploceli* n.sp.

Fig. 7. A trophozoite.
Fig. 8. An early schizont.
Fig. 9. A mature schizont showing 8 nuclei.
Fig. 10. An early gametocyte.
Figs. 11 & 12. Female gametocytes.
Fig. 13. A male gametocyte.

Figs. 14–21. *Plasmodium pericrocoti* n.sp.

Fig. 14. A trophozoite.
Fig. 15. A corpuscle showing multiple infection by the parasites.
Figs. 16 & 17. Early schizonts.
Fig. 18. A schizont showing pigment granules.
Fig. 19. A mature schizont showing 16 nuclei. Note the concentration of the pigment granules into a small dense mass.
Fig. 20. A female gametocyte.
Fig. 21. A male gametocyte.

VEGETATIVE PROPAGATION OF *PIPER BETLE* IN THE CENTRAL PROVINCES

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Received March 14, 1945

Readymade 'seeds' or cuttings of *Piper betle* (*Pan*) are not sold as such in markets. The customers have to purchase the full length of pan vines to make the 'seeds' themselves out of them. In the Central Provinces it is a common practice of the *baris* (pan growers) to use one or two top-most cuttings, each consisting of four to six internodes measuring from 12 to 18 inches in length. After taking the required number of cuttings from a vine the rest of it is discarded though the leaves from them are removed and sold. Before planting is carried out the leaves from the lower half of the first two top-most cuttings are also removed and marketed. The vines for 'seed' purposes are available at an average rate of 1,000 per sixteen rupees. Cuttings are generally planted in pairs and 1,40,000 pieces are required for an acre of land. Before the advent of foot-rot disease (*Phytopthhora parasitica* var. *piperina* Dast.) in an epidemic form the pan 'seeds' (cuttings) were in abundance and cheap. At present when hardly a few gardens are left undestroyed by foot-rot disease and when it has become extremely difficult to procure 'seeds' even at exhorbitant prices, the *baris* have adopted the practice of taking as many as six cuttings from each vine, very often with poor yield and little or no income.

It is a general saying that the cuttings which are taken from the upper parts of vines give a higher yield of pan leaves than those taken from the lower ones. But as the cuttings which are planted in a pan garden are always of a mixed nature, it has not been found possible to verify the statement and to come to a conclusion about the yield of the cuttings taken in a successive order from tip downwards of a vine nor it has been possible to recommend the number of desirable cuttings to be taken from each vine so that the maximum yield and income may be obtained. A systematic study of the problem was taken up and the data have yielded valuable results.

The seeds or cuttings of *kapuri* variety of pan were taken from two years old vines as they happen to be the best in their growth. Each vine was cut from tip to base into twelve pieces, each piece consisting of five internodes

and measuring from 12 to 15 inches in length. In the text these pieces will be referred to in order of succession from tip downwards as 1st, 2nd,....and 12th cuttings respectively. It is a common practice of *baris* to have generally three leaves with each cutting. Following the above practice 1st to 4th cuttings had three leaves each, 5th and 6th cuttings had two leaves each and 7th to 12th cuttings without any leaves as they had none on them.

A rectangular piece of land measuring 100 by 7 feet in dimensions was divided into four equal plots, each measuring 25 × 7 feet. Each plot was further divided into 12 rows, each seven feet long. One row in each plot was selected at random for each of the twelve types of cuttings. The experiment was replicated four times. All the four plots were manured with linseed oilcake once every month from February to September at the rate of 250 lb. per acre per application according to the existing practice of manuring of pan gardens.

The plantation was carried out on the 30th September 1942. Germination started in the 2nd week and continued till the 12th week. 1st, 2nd and 3rd cuttings took longer period for germination than the rest, the best germinations in the former being in the 7th and 8th weeks while in the latter in the 2nd and 3rd weeks. Later it was observed that majority of 5th to 12th cuttings did not survive longer than three months after germination due to poor development of root system (Table I). The mortality due to *Phytophthora parasitica* var. *piperina* Dast. causing foot-rot was also comparatively higher in 5th to 12th cuttings than the rest, the least susceptible being the 3rd cutting.

A record of the linear growth of the pan vines from all the cuttings was maintained and is given in Table II. It will be observed that the highest rate of growth has been found in the 1st cutting with progressively lesser rate of growth in the succeeding cuttings. The growth rate was very poor in the 9th, 10th, 11th and 12th cuttings.

The average yields of betel leaves per plant picked during the first year of the experiment are given in Table III. The leaves were picked and graded according to their size and market value under three heads: (1) *Bade pan* (large leaves), (2) *Khilli* (small leaves) and (3) *Chote khilli* (very small leaves). *Bade pan* are sold at an average rate of 3,000 per rupee, *Khilli* at the rate of 16,000 per rupee and *Chote khilli* have no market value and are thrown away. Yield of *Bade pan* was highest in the 1st, 2nd and 3rd cuttings, low in the 4th cuttings, lower in the 5th and 6th cuttings, least in the 7th cutting and nil in the 8th to 12th cuttings. 5th to 8th cuttings yielded more of *Khilli* while the last four cuttings, 9th to 12th, yielded only *Chote khilli.*

The yield of pan leaves from each type of cutting and the income from their sale proceeds per acre for the first year and for the duration of five years of a pan garden are given in Table IV. It has already been mentioned that the pan "seeds" or cuttings are not sold as such in market but the customers have to purchase the full length of the vines and make seeds out of them. If only one 'seed' is taken from each vine the cost of 'seeds' will amount to Rs. 1,714, the cost of construction will amount to Rs. 800 and the cost

TABLE I

Mortality in pan vines due to foot-rot disease and poor root system

Cutting No.	No. of cuttings germinated out of 100 planted	No. of cuttings died within three months due to poor root system	No. of vines infected with foot-rot							Total No. of vines dead on 30-9-43	Percentage of mortality due to foot-rot and poor development of root-system
			31-3-43	30-4-43	31-5-43	30-6-43	31-7-43	31-8-43	30-9-43		
1st	83	..	4	..	..	..	1	3	14	22	26·5
2nd	94	..	1	3	..	..	..	7	9	20	21·2
3rd	97	..	3	1	..	..	9	2	1	16	16·4
4th	98	..	2	..	..	..	3	17	6	28	28·5
5th	97	17	5	..	..	..	13	27	7	69	71·1
6th	100	12	1	2	..	..	4	12	19	50	50·0
7th	98	49	..	1	..	..	23	5	7	85	86·7
8th	100	53	7	1	..	..	11	1	7	80	80·0
9th	93	47	4	..	..	..	9	4	2	66	70·9
10th	100	58	..	2	..	..	17	19	3	99	99·0
11th	97	43	2	4	..	..	8	22	9	88	90·7
12th	99	62	2	..	..	..	13	9	6	92	92·2

TABLE II

Average linear growth of the vines

Cutting No.	Dates of measurement									
	30-11-42	31-12-42	31-1-43	28-2-43	31-3-43	30-4-43	31-5-43	30-6-43	31-7-43	31-8-43	30-9-43
1st	0′ 6″	0′ 6″	1′ 11″	2′ 4″	2′ 4″	4′ 4″	5′ 6″	6′ 5″	7′ 10″	9′ 3″	10′ 10″
2nd	0′ 6″	0′ 6″	1′ 8″	2′ 0″	2′ 10″	3′ 9″	4′ 8″	5′ 4″	6′ 3″	8′ 0″	9′ 11″
3rd	0′ 6″	0′ 6″	1′ 3″	1′ 3″	2′ 4″	3′ 4″	4′ 6″	5′ 3″	6′ 1″	8′ 0″	9′ 9″
4th	0′ 5″	0′ 6″	0′ 6″	0′ 7″	1′ 2″	1′ 7″	2′ 6″	3′ 3″	4′ 5″	6′ 8″	8′ 8″
5th	0′ 6″	0′ 6″	0′ 6″	0′ 7″	1′ 1″	1′ 2″	2′ 1″	2′ 7″	3′ 10″	5′ 7″	7′ 2″
6th	0′ 6″	0′ 6″	0′ 6″	0′ 7″	0′ 11″	1′ 2″	1′ 11″	2′ 5″	3′ 4″	4′ 11″	6′ 4″
7th	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 9″	0′ 9″	1′ 9″	2′ 10″	4′ 1″
8th	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 8″	0′ 9″	1′ 8″	2′ 10″	4′ 0″
9th	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 8″	0′ 8″	0′ 11″	1′ 3″	1′ 10″
10th	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 8″	0′ 8″	0′ 11″	1′ 3″	1′ 9″
11th	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 8″	0′ 8″	0′ 11″	1′ 3″	1′ 9″
12th	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 6″	0′ 8″	0′ 8″	0′ 11″	1′ 3″	1′ 9″

TABLE III

Average yield of betel leaves per plant per annum

Cutting No.	1st picking March		2nd picking May		3rd picking July		4th picking September			Total yield per plant per annum					
										Bade pan		*Khilli*		*Chote khilli*	
	Bade pan	*Khilli*	*Bade pan*	*Khilli*	*Bade pan*	*Khilli*	*Bade pan*	*Khilli*	*Chote khilli*	Total No.	Per-centage	Total No.	Per-centage	Total No.	Per-centage
1st	6	2	19	8	21	7	32	13	..	78	72·2	30	27·8	..	..
2nd	5	3	16	8	20	11	22	9	..	63	67·1	31	32·9	..	..
3rd	5	3	14	7	20	10	25	12	..	64	67·3	31	32·6	..	..
4th	3	..	4	3	9	7	15	10	..	31	60·8	20	39·2	..	..
5th	..	..	2	5	7	9	9	12	..	18	40·0	27	60·0	..	..
6th	..	..	3	6	5	9	10	14	..	18	38·2	29	61·7	..	..
7th	..	..	..	..	..	9	2	14	..	8	8·0	23	92·0	..	..
8th	..	..	..	..	..	9	..	18	..	..	..	27	10·0	..	..
9th	..	..	..	..	..	..	..	..	21	..	..	..	..	21	100
10th	..	..	..	..	..	..	..	..	18	..	..	..	..	18	100
11th	..	..	..	..	..	..	..	..	13	..	..	..	..	13	100
12th	..	..	..	..	..	..	..	..	13	..	..	..	..	13	100

TABLE IV

Yield and income from each type of cutting

Cutting No.	Yield of *Bade pan* per acre per annum	Income from *Bade pan* per acre per annum (in rupees)	Yield of *Khilli* per acre per annum	Income from *Khilli* per acre per annum (in rupees)	*Yield of *Chote khilli* per acre per annum	Total income per acre per annum (in rupees)	Income over expenditure during first year (in rupees)	Total net profit during five years (in rupees)
1st	1,09,20,000	3,640	42,00,000	262	..	3,902	+ 188	+10,996
2nd	88,20,000	2,940	43,40,400	271	..	3,211	− 503	+ 8,341
3rd	89,69,000	2,986	43,40,400	271	..	3,257	− 457	+ 7,771
4th	43,40,000	1,446	28,00,000	175	..	1,621	−2,093	− 409
5th	25,20,000	840	37,80,000	236	..	1,076	−2,638	− 2,142
6th	25,20,000	840	40,60,000	253	..	1,093	−2,621	− 3,0 9
7th	2,80,000	93	32,20,000	201	..	294	−3,420	− 7,044
8th	..	..	37,80,000	236	..	236	−3,478	− 7,334
9th	..	..	..	..	29,40,000	nil	−3,714	− 8,514
10th	..	..	..	..	25,20,000	,,	−3,714	− 8,514
11th	..	..	..	..	18,20,000	,,	−3,714	− 8,514
12th	..	..	..	..	18,20,000	,,	−3,714	− 8,514

* No income is derived by the yield of *Chote khilli*

of cultivation per year will come to Rs. 1,200. Thus during the first year each garden will cost Rs. 3,714 and on subsequent years only Rs. 1,200. To avoid foot-rot, leaf-rot and other diseases a pan garden should always be dismantled after five years of plantation and this has of late been generally practised in this province. Therefore the recurring and non-recurring

expenditure of the garden for five years will amount to Rs. 8,514, yielding a net income of Rs. 10,996 from the first cutting, Rs. 8,341 from the 2nd and Rs. 7,771 from the 3rd cutting. If other than first three cuttings are planted there will be a definite loss to the growers. During the first year only the 1st cuttings will show the profit.

The above, however, is not the general practice. These days the growers always plant several cuttings from each vine. It will be seen from Table V

TABLE V

Yield and income from mixed cuttings

Cost of construction of a pan garden per acre—Rs. 800.
Cost of cultivation per acre per annum—Rs. 1,200.
Total expenditure during 2nd to 5th years—Rs. 1,200.

Cutting No.	Cost of cuttings per acre (in rupees)	Total expenditure per acre for the first year (in rupees)	Total income per acre for the first yrar (in rupees)	Income over expenditure for the first year (in rupees)	Income over expenditure for five years
1st	1,714	3,714	3,902	+188	+10,996
1st and 2nd	908	2,908	3,556	+648	+10,072
1st, 2nd and 3rd	628	2,628	2,456	+828	+ 9,752
1st to 4th	496	2,496	2,997	+501	+ 7,689
1st to 5th	398	2,398	2,613	+215	+ 5,867
1st to 6th	330	2,330	2,360	+ 30	+ 4,670
1st to 7th	286	2,286	2,064	−222	+ 3,234
1st to 8th	250	2,250	1,836	−414	+ 2,130
1st to 9th	220	2,220	1,632	−588	+ 1,130
1st to 10th	198	2,198	1,469	−729	+ 347
1st to 11th	180	2,180	1,335	−845	− 305
1st to 12th	164	2,164	1,224	−940	− 844

that the highest income during the life time (5 years) of a pan garden is obtained when the 1st cutting is taken, closely followed by 1st and 2nd, and 1st, 2nd and 3rd cuttings. If 1st and 2nd cuttings are taken the initial expenditure which is rather of importance from the cultivators' point of view is lessened by Rs. 806 but if 1st, 2nd and 3rd cuttings are taken from each vine then the initial expenditure is further lessened by Rs. 1,086 without any significant loss to the cultivators.

The above experiments have conclusively shown that only first three cuttings should be taken from each vine. This procedure will minimise the incidence of diseases, specially foot-rot and will pay handsomely the cultivators with only an initial expenditure of Rs. 2,628 per acre.

SUMMARY

1. Out of the twelve cuttings from each vine the first three took comparatively a longer period for germination than the rest.

2. Mortality due to *Phytophthora parasitica* var. *piperina* was highest in 5th to 12th cuttings, the least susceptible being the 3rd cutting.

3. Highest rate of growth was observed in the first cutting with progressively lesser rate in the succeeding ones.

4. Yield of leaves was greatest in the first three cuttings.

5. Incidence of foot-rot disease was significantly minimised when only the first three cuttings from each vine were used for propagation.

THE MODE OF ACTION OF POTASSIUM ON UNSTRIATED MUSCLE

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Received April 4, 1945

The action of potassium appears to depend upon a difference in its concentration within and without the fibres, both in the unstriated muscle (Singh, 1939) and in the striated muscle (Wright, 1942). Yet it must have other actions also, dependent upon its action on the cell membrance, permeability, etc.; it may have calcium-like effects. In this paper an attempt has been made to elucidate some of its actions on the excitability of unstriated muscle.

Results

Potassium inside the fibres.—According to Straub, muscarine acts on the heart only as it penetrates into the muscle cells, and once having arrived in the interior, has no further action. In this respect, potassium acts similarly. The concentration of potassium inside the fibres is greater than that outside, and it does not cause diminution in excitability. This may be related to greater concentration of calcium inside the fibres, which antagonises the action of potassium. The same concentration of potassium outside causes inexcitability, which is antagonised by increase in the concentration of calcium.

As calcium and potassium mutually antagonise the depressant action of each other within certain limits with resulting increase in excitability, it follows that the excitability to alternating current will increase, if the osmotic pressure of the saline is increased upto a certain limit. This limit is found to be 1·4 times normal, after which increase in the concentration of the ions inside the fibres begins to exert a depressant effect. The effect of electrolyte-free medium (Singh, 1944 *a*) shows that potassium inside the fibres becomes toxic, if the concentration of the ions outside the fibre is decreased, thus increasing its relative concentrations inside the fibres. The depressant effect of increase in osmotic pressure of the saline would presumably be due to a similar cause.

The depression of excitability if the osmotic pressure of the saline is increased is partly due to increase in the concentration of the ions inside the fibres, and partly due to the substance used in increasing the tonicity

of the saline. The latter action is shown by the fact that the various sugars sodium salts (chloride, bromide, nitrate, idoide and thiocynate) and chlorides (lithium, sodium, ammonium and potassium), do not produce identical results, if used in increasing the tonicity of the saline. They produce osmotic results as well as their own individual actions; the former action is shown by the fact that the sodium chloride and sucrose, which are chemically unrelated produce identical results, when used in increasing the tonicity of the saline.

The relative concentration of ions inside the fibres is increased if the external medium is made sodium deficient by replacement with sucrose. The excitability to alternating current in the frog stomach is at first decreased and then increased. The same results are produced if the osmotic pressure of the saline is increased by adding sucrose or sodium chloride at pH 7. The similarity of the action of sodium deficient and hpyertonic solutions, is probably due to the fact, that in both instances, the concentrations of the ions is relatively increased in the interior of the muscle fibres.

When sodium chloride is added, then the relative concentration of all ions is the same outside and inside, so that the effect of increase of osmotic pressure are not due to increase in the concentration of all the ions inside the fibres, but of those more potent than sodium or chloride, that is, potassium or calcium. As increase in osmotic pressure of the saline causes contraction, the effects of such increase must be due to potassium, as calcium has an inhibitory effect.

When the osmotic pressure of the saline is incresed, both in *Mytilus* and frog stomach muscles, the excitability to alternating current may at first be decreased and then increased. This suggests that potassium inside the fibres, above its normal concentrations, either causes contraction or depression in excitability, just as it does outside the fibres. The subsequent increase in excitability shows an increase in the concentration of the antagonistic factor, probably calcium. The inhibitory effect of increase in osmotic pressure would therefore be due to increase in the concentration of potassium, as well as calcium inside the fibres; the increase in concentration of the former will cause a secondary increase in the concentration of the latter, due to adaptation (Singh, 1944 *b*). It is so interesting, that increase in the concentration of potassium inside and outside the fibres causes some similar results. Thus when the concentration of potassium is increased outside, the excitability to alternating current at first decreases and then increases, and finally decreases. These are also the effects of increase in osmotic pressure of the saline.

The frog stomach and the heart can be stimulated electrically in the electrolyte-free medium (Singh, Sehra and Mrs. Singh, 1945 *a*, *b*), as well as by increase in osmotic pressure of the saline to 1·4 times normal. This shows that this contraction as well as spontaneous contractions are due to potassium inside the fibres. The occurence of spontaneous contractions suggests, therefore, that potassium is periodically liberated from some labile combination in the cells.

As the excitability to potassium outside the fibres depends upon the concentration of potassium inside, and as the latter can be varied by changes in osmotic pressure, this suggests that potassium exists in two forms within the cells, one free and the other combined, because the changes in the concentration of the active potassium only would affect the excitability to potassium outside the fibres, if this excitability is dependent upon a difference in concentration of potassium on two sides of the muscle membrane. The free potassium inside the fibres, therefore, determines, what may be termed as "internal excitatory state", analogous to the central excitatory state in the neurone. The internal excitatory state will increase the excitability to the excitation produced by ions inside the muscle fibres, but decrease that to ions outside.

In the frog stomach, the adaptation occurs to alternating current in the electrolyte-free medium. This shows that the factor of adaptation occurs inside the fibres, that is due to liberation of calcium. Calcium, therefore, also probably exists in a combined and free form inside the cells (Singh, 1944), and the free or labile calcium inside the fibres, therefore determines the "internal inhibitory state", analogous to the central inhibitory state in the neurone.

Adaptation to excitation occurs, by increase in the inhibitory state, while adaptation to inhibition occurs by increase in the excitatory state (Singh, 1944 *b*). The increase in the excitatory state after inhibition is responsible for the withdrawl contractions, which are the same phenomenon as rebound in the spinal reflexes. The above phenomenon are similar to those in the neurone. In the latter when the P effect predominates, a further P effect is less easily produced than the N effect; the production of the P is said to be occluded and that of N facilitated and *vice versa*. Just as with knee jerk a succession of inhibitions and rebounds after adrenaline may cause dog stomach to enter into a tonic contraction. The above explains a very common phenomenon in plain muscle. If a substance depresses the excitability, its removal causes an increase in excitability before return to the normal. Similarly if a substance increases the excitability, there may be a depression

of excitability before return to the normal; this latter phenomenon is not always found. The above also accounts for the supernormal states in the plain muscle.

The refractory period of the muscle is partly due to this inhibitory state. This is shown by the fact that in the frog heart complete tetanus is not produced if the concentration of calcium is increased. It has been explained that the length of the refractory period prevents the frog heart from tetanising, so that if the concentration of calcium is decreased, then the refractory period must get shorter. The reason why then acetylcholine favours tetanus in the frog heart is that adaptation to inhibition is produced by increase in the excitatory state. Spontaneous contractions are then due to the interaction between the excitatory and inhibitory states, and also accounts for the fact that a relaxed muscle may contract and a contracted muscle relax on stimulation.

When *Mytilus* muscle, which is comparatively less sensitive to potassium, is stimulated with the latter or adrenaline, it may become inexcitable after two or three stimulations, owing to the development of an inhibitory state. The above experiment shows that the inhibitory state persists longer, and rises more slowly than the excitatory state (Singh, 1944 *b*), as happens in the neurone. Fatigue is therefore due to accumulation or "facillitation" of the inhibitory state; it is therefore identical with adaptation and refractory period. As the refractory period varies in different tissues, it is not due to exhaustion of "food energy".

It is generally found that whatever diminishes the excitability of unstriated muscle, also increases the latent period, and diminishes the rate of rise of tension. But the opposite holds at high temperatures, when the excitability diminishes, but the refractory period also deminishes. This diminution in the refractory period at high temperatures is presumably due to increase in the rate of development of the excitatory state and the rapid adaption or fatigue to increase in the rate of the inhibitory state. The refractory period would therefore be due to the resting or threshold inhibitory state. In *Mytilus* muscle excess of magnesium may increase the latent period several hundredfolds, without appreciably altering the rate of rise of tension of the contraction produced by alternating current.

In unstriated muscle there may be both summation of excitatory and inhibitory states, and also subliminal excitation (Singh, 1939) or inhibition.

The action of distilled water on the frog stomach (Singh, 1944) suggests that normal tone and viscosity of the muscle are due to combination of ions inside the fibres with myosin. In the electrolyte-free medium relaxa-

tion is slow, so that ions inside the fibres also produce tonic contraction. In sucrose solution, the muscle contracts, so that the factor causing tonic contraction resides inside the fibres.

The greater tone and viscosity of the unstriated muscle appears to be related to its greater sodium content (Singh, 1938). It therefore appears that sodium chloride inside the fibres antagonises the action of calcium, as it does outside the fibres. Like potassium and calcium, sodium probably exists in the bound and free form. Tonic contractions such as slow relaxation are then probably due to mobilisation of sodium, twitch, to mobilisation of potassium, and adaptation to mobilisation of calcium inside the fibres. Tonic contraction may also be due to ions outside the fibres; the sodium inside the fibres, may then be said to determine the "tonic and viscous state".

Potassium outside the fibres.—The contracture which is produced by increasing the concentration of potassium in the saline is diminished by increase in the osmotic pressure of the saline, which increases the concentration of ions within the muscle fibres. The action of potassium thus appears to be antagonistic on two sides of the muscle membrane. This veiw is supported by the further observation, that tone which is produced in the frog stomach by increase of osmotic pressure, is diminished by increase in the concentration of potassium in the saline within certain limits. Thus tone produced by increase in the osmotic pressure of the saline to twice the normal, is diminished if the concentration of potassium is increased to five times the normal the excitability to alternating current increasing at the same time (Fig. 1).

The maximum excitability of the muscle, thus depends upon a certain optimum ratio of potassium without and within the fibres. If this ratio is disturbed then the excitability diminishes and a contracture develops. This ratio can be disturbed in four ways:

1. Increase in the concentration of potassium within the muscle fibres; thus occurs when the osmotic pressure of the saline is increased.

2. Decrease in the concentration of the ions inside the fibres; this occurs when the osmotic pressure of the saline is decreased.

3. An increase in the concentration of potassium outside the fibres.

4. A decrease in the concentration of potassium outside the fibres; this also occurs in familial periodic paralysis (Aitken, Allot, Caslteden, and Walker, 1937).

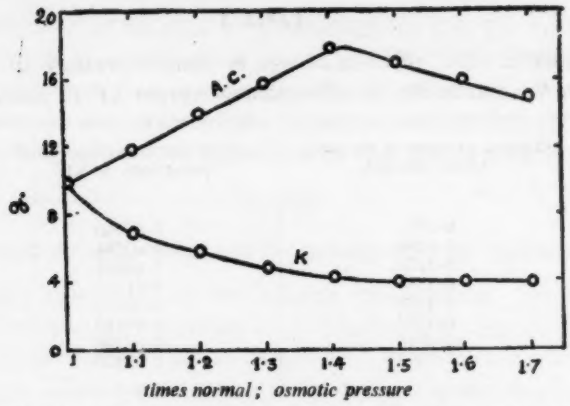

FIG. 1 A. Frog stomach : The effect of osmotic pressure on tone produced by replacement of 20% of the sodium of the saline by potassium.

AC = tension produced by alternating current ; K = tone produced by potassium.

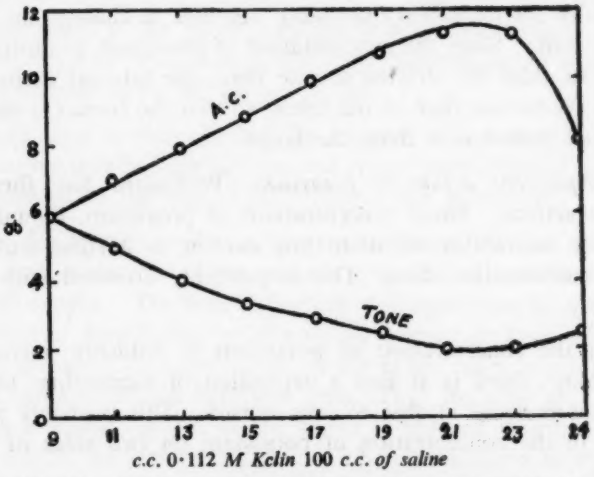

FIG. 1 B. Frog stomach : effect of potassium on tone produced by doubling the osmotic pressure of the saline by sucrose.

If the above view is correct, then the optimum concentration of potassium in the saline for excitability should vary with the osmotic pressure of the saline. It has been found (6 experiments), that with the decrease of osmotic pressure, the optimum concentration of potassium required for excitability to electric current decreases; the opposite happens with increase in osmotic pressure (Table I). These results suggest

TABLE I

Frog stomach. The effect of change in osmotic pressure of the saline on the excitability to alternating current 8V/10 Seconds

Osmotic pressure of the saline Molar strength	Optimum concentration of potassium, M KCl
0·0672	0·00560
0·0896	0·00784
0·1120	0·01008
0·1344	0·01232
0·1568	0·01456
0·1792	0·01780
0·2016	0·02240
0·2240	0·02520

that one of the actions of potassium is dependent upon its difference in concentration on two sides of the muscle membrane.

Under normal circumstances, a change in the optimum concentration of potassium for excitability probably signifies a change in the internal excitatory state. Since the concentration of potassium in unstriated muscle fibres is less than the striated muscle fibre, the internal excitatory state is greater in the former than in the latter, so that the former is more excitable to electrical stimulation than the latter.

The depressant action of potassium.—Potassium has three kinds of depressant actions. Small concentrations of potassium, diminish tone and increase the excitability to alternating current in *Mytilus* muscle. This is clearly a calcium-like effect. This is probably attended with decrease in permeability.

When the concentration of potassium is suddenly increased in the *Mytilus* saline, there is at first a depression of excitability to alternating current, but increase in that to ions outside. This probably produced by difference in the concentration of potassium on two sides of the muscle membrane.

The increase in excitability is followed by a lasting depression to all forms of excitability. This kind of depression is produced by all abnormal substances when present in excess in the saline. Tone also decreases though tonic contractions by potassium also depresses all forms of excitability. This is antagonised by calcium.

If potassium is in great excess in *Mytilus* saline, then all forms of excitability and tone are abolished; the impedance is greatly decreased,

This is probably due to increase in permeability so that for normal excitability an intermediate permeability is necessary. From the above, it appears that potassium may produce its effect (1) by virtue of a difference of concentration on two sides of the muscle membrane, (2) by action on the muscle membrane, and (3) by action on the muscle colloids.

Tonus

In unstriated muscle, tonus can be produced in the following ways:—

1. *Intrinsic tone.*—An isolated muscle shows tonus, which does not appear to use oxygen, as it appears to be unaffected by cessation of oxygen supply; in fact the muscle dies in that state. Plain muscle always dies in a state of tonus. This tone appears to be due to the action of ions in the muscle fibres on the muscle protein, as shown by the action of distilled water. In water, the muscle though alive, actively elongates; this can be ascribed to diminution of concentration of ions inside the muscle fibres, as the muscle gains water. An isolated muscle preserves the property of elongation after death if the latter is caused to occur naturally in saline; it appears that in this way death causes the least disturbance in protoplasm, and myosin preserves some of its properties.

The above tone is characterised by absence of spontaneous contractions; the viscosity may be high or low, and is not much affected by increase in osmotic pressure of the saline; inhibition is not prominent.

2. *Tonus due to saline.*—An isolated unstriated muscle may show considerable tone, dependent upon oxygen supply; the tone diminishes in the absence of oxygen. The tone is marked with spontaneous contractions and low viscosity. Excitability to alternating current is low; *inhibition is facilitated.* It is due to the constituents of the saline, such as sodium chloride, calcium, potassium and hydrogen ions. Thus the tone may diminish if the sodium chloride content of the saline is reduced, showing that the latter salt is one of the stimulating factors. Again reduction in the sodium chloride content may only be effective if the potassium is absent, showing that the latter is also stimulating agent; the effect of potassium may be reverse. The reduction in the sodium chloride content of the saline may be effective only in alkaline solution, showing that the hydrogen ions are also stimulating agents; their action may be reverse of the above. Calcium has both stimulating and inhibitory properties.

3. *Tonus due to stimulating substances.*—This is due to the action of stimulants. The oxygen consumption may or may not increase; the viscosity

is high. Spontaneous contractions may be absent. The excitability is depressed; *inhibition is antagonised.*

4. *Tonus in sucrose solution.*—This is due to unbalanced action of ions inside the fibres. In frog stomach it is reduced by reducing the osmotic pressure. Spontaneous contractions are absent, excitability is depressed and excitation and inhibition are abolished.

5. *Tonus due to hypertonic saline.*—This is due to increase in the concentration of potassium inside the fibres; spontaneous contractions are increased. Inhibition is antagonised.

6. *Tonus due to hypotonic saline.*—This is due to the increased action of ions outside; inhibition is increased and viscosity is high.

Effect of potassium on tone.—Small concentrations of potassium (normally present in saline) have an inhibitory influence. In isotonic glucose in *Mytilus* muscle, and isotonic sucrose in frog stomach, they have a stimulating action. Larger concentration of potassium have an inhibitory action. This latter action would be due to their antagonising the ions inside by restoring somewhat the ionic in balance within and without the fibres. With increase in the concentration of potassium, the excitability to alternating current is not increased, as it does when potassium antagonises the tone produced by increase in osmotic pressure. It would appear that ions in the saline have a protective action against the depressant action of potassium. In *Mytilus* muscle, if the osmotic pressure is increased by sodium chloride then increase of excitability may result (Fig. 2); the latter action is more

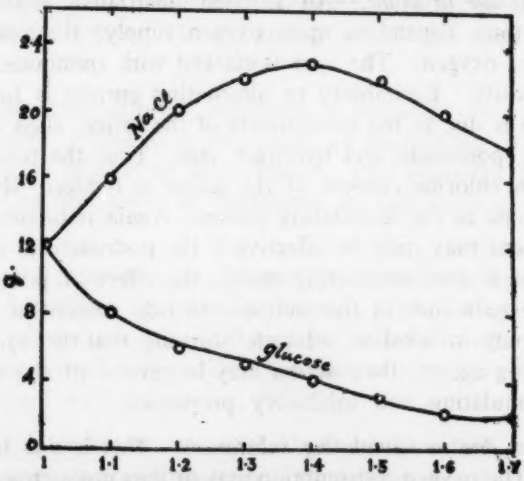

FIG. 2. *Mytilus* muscle : Effect of increase in osmotic pressure by NaCl and glucose on tension produced by alternating current. Results are very variable, however,

marked in muscles that have been obtained from animals stored in cold, and thus contain more sodium. It thus appears that sodium chloride protects the muscle membrane from the action of potassium. In frog stomach also, sodium chloride is more efficacious than sucrose at pH 8.

Both frog heart as well as frog stomach remain irritable in the electrolyte free medium, but skeletal muscle does not. This is probably due to the fact that the former two contain more sodium than the latter. In the guinea pig uterus, removal of potassium from the saline may cause increase or decrease of tone. The former action shows that as in *Mytilus* muscle it has an inhibitory action. The former action occurs in alkaline and the latter in acid solutions. After 10 minutes in both instances, reintroduction of potassium causes marked inhibition. These results can be explained as previously (Singh, 1944 *b*) that potassium suppresses the liberation of calcium or the inhibitory state. In alkaline solutions, the liberation of calcium is not sufficient to overcome the increase in tone but in acid solutions, the hydrogen ions reinforce the action of liberated calcium.

In the frog stomach, small concentrations of potassium cause contraction by antagonising the action of sodium chloride, but their stimulating action in isotonic sucrose solution is not understood.

Effect of calcium.—Calcium in small concentrations decreases tone produced by (1) ions outside, (2) isotonic sucrose or increase in osmotic pressure of the saline, that is ions inside. This is in agreement with the suggestion that it produces adaptation. In isotonic sucrose solution, small concentrations of potassium, cause contraction, but calcium causes relaxation. In frog saline, both have a stimulatory effect, calcium and potassium, in this instance antagonising the inhibitory effect of the sodium chloride. By same action in *Mytilus* muscle, they produce relaxation. The inhibitory effect of sodium chloride is like that of calcium.

In guinea pig uterus, withdrawl of calcium causes decrease of tone, both in alkaline (pH 8, borate buffer), and in acid (phosphate buffer) solutions. Reintroduction of calcium causes contraction in alkaline solutions, but in acid solutions may cause a preliminary inhibition. The cause of this is probably the same as that of potassium inhibition, that is liberation of calcium on withdrawal of calcium (Singh, 1944 *b*); for the same reason the diminution of tone on withdrawl of calcium is less in acid solutions than in alkaline soultions. The liberated calcium in acid solutions would partly compensate for the withdrawal of calcium.

Summary and Conclusions

(1) The action of potassium, calcium and sodium suggest that they exist in the combined and free form in the unstriated muscle, they respectively determine the excitatory, inhibitory and tonic and viscous states in the muscle.

(2) One of the actions of potassium is due to its difference in concentration on the two sides of the muscle membrane. This is shown by the fact that the optimum concentration of potassium in the saline for excitability depends upon its concentration inside the fibres. For maximum excitability there is an optimum ratio of potassium on two sides of the muscle membrane.

(3) The second action of potassium is probably on the cell membrane as it is antagonised by calcium.

(4) A third action of potassium is probably on the muscle colloid as it affects the viscosity of the muscle.

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SOME FOSSIL LEAVES OF THE SALICACEÆ FROM NINGAL NULLAH AND LAREDURA, PIR PANJAL, WITH A NOTE ON THE SIGNIFICANCE OF TEMPERATE SPECIES IN THE PLEISTOCENE FLORA OF KASHMIR

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Received July 5, 1945

(With Four Plates)

(Communicated by Prof. Birbal Sahni, F.A.SC., F.R.S.)

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Introduction

Nearly eight years ago, in the summer of 1935, Dr. R. R. Stewart of the Gordon College, Rawalpindi, made a collection of about 600 plant-fossils from the Lower Karewa deposits of Kashmir exposed at Ningal Nullah (alt. 9,000 ft.; lat. 34° 4′; long. 74° 19′) in the neighbourhood of Botapathri (Survey of India Map 43 J/8), a summer abode of Kashmiri shepherds on the northern slopes of the Pir Panjal Range. The fossiliferous beds, which

are assigned the Lower Pleistocene Age by De Terra and Paterson (1939) occur in the form of dome-shaped low grassy mounds stretching for over a mile from the right bank of the Ningal Nullah a perennial stream, which has its origin somewhere on the high peaks of the Pir Panjal. Numerous small rivulets, and artificial water channels have dissected their way through the clayey deposits and have furnished many plant-bearing outcrops for easy collection of fossil material. Dr. Stewart's collections were made from a few spots along two water channels.

Some years later, in 1941, the author visited this locality and discovered another fossiliferous stratum running parallel to the Ningal Nullah stream at a distance of over a furlong from its right bank. This stratum is exposed in the dried bed of a hill stream, which has cut through the beds as deep as 8–10 feet at some places. Small blocks of clay can be easily excavated for splitting, but larger blocks could not be extracted on account of the friable nature of the clay.

The plant material gathered both by Dr. Stewart and myself from this locality consists of leaf impressions embedded in a loosely-set sandy clay intermixed with small quantities of loam. The clays are very regularly bedded and split out neatly along the plane of bedding into thin layers, often as fine as a centimetre in thickness. Most of the larger leaves split out fragmentary on account of the friable nature of the clay, and small leaves being rather uncommon, the material, therefore, abounds in incomplete leaves.

The entire material is under investigation at Lucknow under the guidance of Professor B. Sahni, F.R.S., and a flora of 62 genera and 122 species distributed over 34 families of Angiosperms was reported by the author (Puri, 1942, pp. 222–24) in 1941. The present paper is devoted to the description of fossil leaves of the Salicaceæ in this material.

In addition to the Ningal Nullah material this paper also includes a description of two more leaves of *Salix* sp., collected by the author in 1940 from Laredura (alt. 6,000 ft.; 34° 7′ N.; 74° 21′ E.), a small village near Dangarpur (Survey of India Map 43 J/8) on the northern slopes of the Pir Panjal.

Condition of the Material

The fossil leaves from Ningal Nullah are impressions mostly from skeletal net works of veins; and no trace of organic matter of a leaf is preserved in any of them. Our experience in the field showed that the freshly split leaves in a dry atmosphere tend to wither away and thin layers of clay

often warp and curve up in sun thereby spoiling the impressions. Therefore, some of them were painted in field with varnish or canada balsam, which proved quite useful in preserving details of venation.

The state of the fossil material often indicates the physical conditions under which the plant parts had been deposited. Applying this principle to our material it may be deduced that the fossil leaves at Ningal Nullah had probably rotted in water for a considerable time before getting deposited in the lake bed. With a view to ascertain roughly the amount of time required for living leaves of the Karewa species to take up similar appearance in venation with the fossil leaves the author tried rotting of living leaves in the laboratory. On keeping herbarium material of living species of the Salicaceæ in a China-tray in muddy water from the garden tank at room teperatures during the month of February 1941 it was found that the leaves had become quite soft after ten days and the upper and lower epidermis layers could be easily peeled off by a gentle rubbing of the leaves between the fingers. The photographs reproduced in Pl. V, Fig. 3 and Pl. VI, Fig. 14 were taken at this stage for comparison with the fossils. Leaves of *Populus ciliata* are a bit tougher and they take a little longer time to soften. A complete rotting of leaves occurs in a month's time when all other tissues excepting the venation skeleton are dissolved out. Fresh leaves of a *Salix* sp. from the garden rotted out comparatively earlier than dried herbarium material.

Incidentally it may be useful to mention here that cuticles of living leaves can be easily prepared in this way and by immersing them in nitric acid and potassium chlorate for an hour or so they may, perhaps, be employed for comparison with fossil cuticles.

This paper has been prepared under the guidance of Professor B. Sahni, F.R.S., to whom I am greatly indebted for invaluable help and stimulating criticism.

I am also grateful to Dr. R. R. Stewart, my old teacher in Systematic Botany, for a loan of his material and for facilities extended to me for work in his Herbarium. As usual I have to thank the Vice-Chancellor, University of the Panjab and Principal Jodh Singh of the Khalsa College, Amritsar, for a research grant from that University and authorities of the Lucknow University for a Research Fellowship. I am glad to acknowledge the help my wife has given me in correcting the proofs.

Systematic List of the Species

Salix Wallichiana Anders.
Salix denticulata Anders.
Salix sp. A
Salix sp. B
Salix sp. C
Populus ciliata Wall.
Populus nigra L.
Populus sp. A.
Populus sp. B

Description

Order: Salicales

Family: Salicaceæ

The family Salicaceæ is represented in the Karewa flora by four well-determined species belonging to two modern genera from Laredura and Ningal Nullah. The species are based on leaves, the majority of which are fragments of various sizes.

The leaves of this family, whether living, or fossils, have some characteristic features, which are remarkably uniform and constant in the two genera and on the basis of macroscopic features such as shape and venation one can recognise and separate willows and poplars from an assemblage of living, or fossil leaves. Though the generic identification is attained with ease, it is comparatively more difficult to distinguish between the different species, some of which have very similar leaves.

In addition to four fully determined species there are three more leaves of *Salix* which could not be specifically determined on account of their fragmentary nature, and two well preserved distinct leaf fragments of *Populus* which do not seem to match any living species of the Himalayas; and hence they are incompletely determined. The family includes in all nine fossil species, five of which belong to *Salix* and four to *Populus*.

Key to the Genera

I. Leaves linear-lanceolate, or linear-oblong, secondaries 15–32 pairs .. .. *Salix* (1)
II. Leaves ovate, secondaries 5–8 pairs .. .. *Populus* (2)

Genus *Salix* Linn.

The genus is represented by two fully determined and three half determined species. There are a few leaves which may belong to either of

the two or to both species, namely, *Salix acmophylla* Boiss. or *Salix viminalis* Linn. Three more specimens, which are nicely preserved leaf fragments, could not be referred to any living species, and are described as *Salix* sp. A, B and C.

All specimens, with the exception of one complete leaf, are fragmentary impressions showing well-preserved venation. Excepting two leaves, which come from the author's collections, all others had been previously heavily varnished by Dr. Stewart in field or laboratory as a result of which the photographs of these did not show up well the details of venation. One complete leaf is found folded on itself at about its middle.

The classification of living willows is a very difficult task, even when the flowers and fruits are present, and the determination of species from fossil leaf fragments is still more difficult, but the author having made a diligent study of the macroscopic characters of living species has been able to prepare the following key for the fossil species, described in this paper:—

KEY TO THE SPECIES

I. Leaves large, oblong-lanceolate, fragment 3·3″ × 7″, secondaries 20 pairs .. .. *Salix Wallichiana* (1)

II. Leaves much smaller .. *Salix* sp. A and *Salix denticulata*

(i) Leaves lanceolate, 4″ × ·5″, secondary nerves 19–20 pairs .. ..*Salix* sp. A (3)

(ii) Leaves oblong, leaf 1·45″ × ·75″, secondaries 9–10 pairs .. .. *Salix denticulata* (2)

(iii) leaves linear, fragment 2·6″ × ·6″

(*a*) Secondaries about 26 pairs .. .. *Salix* sp. B (4)

(*b*) Seconbaries abou 14 pairs .. *Salix* sp. C (5)

(i) *Salix Wallichiana* Anders.

(Plate V, Figs. 1–3)

The leaf fragment, which is a little more than half of a leaf, represents only the basal portion, which measures 3·3″ long by ·65 inch in its broadest part. It is oblanceolate in outline and has a uniform width for most of its length getting slightly narrowed towards its base, which bears a small, thick petiole, measuring ·25 inch in length. The leaf fragment tends to become narrowed also towards the broken apical side and it seems to have been probably acute or acuminate at its tip. The margins are probably entire.

The venation is fine and pinnate-reticulate. A fairly strong midrib starts from the base, and runs straight in the lamina gradually thinning out in the

upper (apical) part. 15 to 20 weak secondaries, which are much thinner than the midrib, arise from the latter on either side at acute angles. In between the two secondaries a single weak secondary, which is very much thinner, and much less conspicuous than the midrib, also arises from it at almost the same angle at which the stronger secondaries shoot out. These weaker secondaries do not reach the margins, but soon break up in the lamina and anastomose with tertiary ribs to form long meshes, which constitute the first framework of reticulations (Pl. V, Fig. 2). The stronger as well as the weaker secondaries do not run straight, but follow an undulating course, as a result of which the area enclosed by two secondaries is not strictly rectangular but becomes unsymmetrical (Pl. V, Fig. 2). The width of the enclosed area all along the course of the laterals, therefore, does not remain uniform. The secondaries tend to run parallel, but they arise in a very irregular manner, sometimes sub-opposite, opposite, or often alternate. At some places a weaker and a stronger secondary rib may anastomose with each other and become one. The secondaries do not as a rule end abruptly in the margin, but run along it for some distance before they get lost or immersed in it (Pl. V, Fig. 2). The meshes of the tertiary reticulations are fairly conspicuous and strong. They are seen in the form of polygonal, or angular meshes (Pl. V, Fig. 2). The finer reticulations are greatly obscured by varnish, and hence they are not brought out satisfactorily even in a five diameter enlarged photograph of a part of the lamina reproduced in Fig. 2.

The fossil leaf, apparently shows some resemblance with leaves of *Woodfordia fruticosa* (Linn.) S. Kurz., a tropical shrub of the Lythraceæ but a closer examination reveals important differences between the two in details of venation, which were pointed out by the author in a paper dealing with a description of a fossil leaf of *Woodfordia fruticosa* (see Puri, 1943, p. 127).

Our fossil leaf is identical in all respects with leaves of *Salix Wallichiana* Anders, one of which is shown in the photograph (Pl. V, Fig. 3) for comparison with the fossil.

Number of specimens.—Ten.

Occurrence.—Ningal Nullah at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collections.—R. R. Stewart, 1935 and G. S. Puri, 1940.

Registered No. of figured specimen.—N. 132.

(2) *Salix elegans* Wall.

(Plate V, Figs. 6, 7 and Plate VI, Fig. 9)

The two fossil leaves belonging to this species are fairly incomplete, being broken from apex and seem to have an oblong outline of the lamina. They gradually narrow down from the middle into a cuneate base and seem to have been acute at the apex. In one specimen (see Pl. VI, Fig. 9) there is a small twig lying close to the base, and at first sight the leaf may seem to be attached to it but a closer examination shows that the twig in question is lying on a higher level than the leaf and does not have any organic connection with it. The margins of both the leaves are entire.

The venation is fine and pinnate-reticulate. A fairly thick midrib follows an almost straight course in the lamina gradually thinning out in the apical part. 9–10 secondaries, which are about one-fourth as thick as the midrib, diverge from it in an opposite, occasionally sub-opposite and rarely alternate manner at almost equal distances from one another (Pl. V, Figs. 6, 7) on either side at angles of approximately 50 to 55 degrees. They run outwards bifurcating below the margins into thin branches, which are seen in a part of the leaf (Pl. V, Fig. 6) enlarged to five diameters in Plate V, Fig. 7. These branches, at some places, are often indistinguishable from tertiary ribs, which also are of the same nature and thickness. The former tend to form close to the margin by anastomosing with branches of the lower and upper secondaries, seen on righthand side in the enlarged photograph (Pl. V, Fig. 7), a series of small semi-circular loops with their convexities facing outwards. Tertiaries arise from the secondaries at different angles and anastomose variously among themselves to form large meshes of irregular shapes and different sizes (Pl. V, Fig. 7). At some places they are obscured by a thick coat of varnish. These meshes further break up into a close network consisting of small and oval, or polygonal meshes, which constitute a finer reticulation seen clearly in the enlarged photograph (Pl. V, Fig. 7) of a part of the leaf.

Our fossil leaves are identical in all respects with living leaves of *Salix elegans* Wall. with which they are, therefore, identified.

Number of specimens.—Two.

Occurrence.—Laredura, at 6,000 ft. and Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collections.—R. R. Stewart, 1935, and G. S. Puri, 1939.

Registered Nos. of figured specimens.—Plate V, Figs. 6, 7 = N 20; Plate VI, Fig. 9 = L 150.

(3) *Salix* sp. A

(Plate V, Figs. 4, 5)

Plate V, Fig. 4 is a natural size photograph of a complete leaf, which measures 1·64 inches long by ·5 inch in the broadest part, a little above the middle, and has a lanceolate outline of the slightly oblique lamina. It narrows down abruptly into a cuneate base and also tapers upwards into an acute or somewhat acuminate apex and possesses entire margins. An irregularly applied thick coating of varnish, which appears in the photograph (Pl. V, Fig. 4) as dark patches along the margins may conceal the true extent of the margins, which are otherwise fairly well marked out in the specimen.

The venation is fine and pinnate-reticulate. A conspicuous midrib runs in the lamina gradually thinning out upwards and lies slightly displaced from its centre thereby dividing the lamina into two slightly asymmetrical halves. 19–20 weak secondaries, inconspicuous and much thinner than the midrib, shoot out at angles of approximately 40 degrees, on either side of the midrib and tend to remain equidistant and run closely in the lamina more or less parallel to one another and become slightly arcuate (Pl. V, Fig. 5) in the upper part of the leaf, where they tend to converge. They do not end directly in the margin but a little below it form a series of loops or sometimes large meshes by curving upwards and inwards and anastomosing with the superior laterals on the same side. Tertiaries are indistinct, largely obscured by the varnish, but here and there, they are visible forming large meshes of different shapes and sizes seen in the photograph (Pl. V, Fig. 5) of a part of the leaf enlarged to five diameters. Finer reticulation also is largely obscured by the varnish and consequently it is not brought out in the photograph.

Our fossil leaf does not seem to resemble any one species of *Salix* though in some features, *e.g.*, shape, size, margins, etc., it may show some resemblance with living leaves of *Salix tetrasperma* Roxb.; but with our present state of knowledge concerning the macroscopic features of living leaves of willows it seems unsafe to specifically determine our fossil leaf with any living species of *Salix*, hence it is described here as Sp. A.

Number of specimens.—Five.

Occurrence.—Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collection.—R. R. Stewart, 1935.

Registered No. of figured specimen.—N 203.

(4) *Salix* sp. B

(Plate VI, Figs. 10, 11)

Plate VI, Fig. 10 is a natural size photograph of a fossil leaf, which is folded on itself at about the middle and is slightly broken at the apical end. It is lanceolate in outline and measures 2·6 inches long by ·6 inch in the broadest part, and slightly narrows downwards into a more or less rounded base bearing a bit of petiole, and possesses entire margins.

The venation is fine and pinnate-reticulate. A fairly stout midrib runs straight in the lamina gradually thinning out towards the apex, and gives off on its either side at acute angles about 26–27 secondaries which are very conspicuous, even though they are not even half as thick as the midrib. The laterals after running straight in the lamina for a short distance from the midrib soon curve upwards and follow an undulating course. Some of the secondaries are pinnate-arcuate, while others are pinnate-looped, which may be seen clearly in a part of the leaf enlarged to five diameters in the photograph represented in Pl. VI, Fig. 11. Sometimes a lower secondary may anastomose near the margin with its superior on the same side or it may run along the latter for some distance and finally anastomose to form irregular loops (Pl. VI, Fig. 11), which in a series seem to form a sort of inframarginal vein running parallel and below the margin (Pl. VI, Fig. 11). There are also one or more weaker secondaries, which arise in between the two stronger secondaries and unlike the latter soon start looping or anastomosing in the lamina with tertiaries fairly down below the margins (Pl. VI, Fig. 11). Tertiaries arise either from the midrib, or from the secondaries and meet halfway in the area enclosed by the two secondaries to form large irregular meshes, which further break up into a well-preserved net work of smaller meshes, which constitute a finer reticulation (Pl. VI, Fig. 11).

Our fossil leaf resembles modern leaves of quite a few species of *Salix*, notably *Salix viminalis* Linn. and *Salix acmophylla* Boiss., both of which have very similar leaves but with our present state of knowledge concerning the macroscopic features of willow leaves it is not possible to specifically determine it, hence it is described here as *Salix* sp. B.

Number of specimens.—One.

Occurrence.—Laredura, at 6,000 ft. in the Pir Panjal Range, Kashmir.

Collections.—G. S. Puri, 1940.

Registered No. of figured specimen.—L 559.

(5) *Salix* sp. C

(Plate V, Fig. 8 and Plate VI, Fig. 12)

Plate V, Fig. 8 is a natural size photograph of a leaf fragment, which measures 2 inches long by ·6 inch in the broadest part. It is broken both from base as well as apex, but evidently it seems to have had a lanceolate outline with an acute apex and narrowed base. The margins are entire.

The venation is fine, and pinnate-reticulate. A fairly stout midrib runs in the fragment slightly thinning out in the upper part and seems to divide the lamina into unequal halves. 13–15 pairs of closely situated, thin secondaries leave the midrib at acute angles and run in an undulating manner towards the margins. There arise in between two strong secondaries one or more weaker secondaries, which do not reach the margins, but either break up into a network of large meshes, hardly distinguishable from tertiary meshes or they unite with other secondaries. Tertiaries usually arise from the midrib and also from the strong as well as weak laterals and variously anastomose to form large meshes, which further break up into a finer reticulation of smaller meshes (Pl. VI, Fig. 12).

The fossil leaf on account of its characteristic venation is identified with the genus *Salix*, but it could not be specifically determined on account of its rsemblance with several species of *Salix* represented in the modern vegetation of Kashmir and neighbouring regions.

Number of specimens.—One.

Occurrence.—Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collector.—G. S. Puri, 1940.

Registered No. of figured specimen.—Loc. 1 N 10.

Genus *Populus*

The genus includes two well-determined species and two others which are distinct and do not match any modern species of the Himalayas. The specimens are impressions of leaves, most of which are imperfectly preserved but on account of their characteristic venation it has been possible to determine them by a comparison with living species of *Populus*.

KEY TO THE SPECIES

I. Leaves large .. .. *Populus ciliata* and *Populus* sp. A
  (i) Secondaries run straight at acute angles .. *P. ciliata* (1)
  (ii) Secondaries arise at almost right angles and run forward forming wide semi-circular curves in the lamina .. *Populus* sp. A (3)

II. Leaves small .. .. .. *Populus nigra, Populus* sp. B

(i) Leaves broad, ovate, or sub-orbicular, wider than long, secondaries 4–5 pairs, wide apart and not equidistant .. *P. nigra* (2)

(ii) Leaf probably ovate, longer than broad, secondaries more than 7 pairs, closer and equidistant .. ..*Populus* sp. B (4)

(i) *Populus ciliata* Wall.

(Plate VI, Fig. 14 and Plate VII, Fig. 15)

Plate VII, Fig. 15 is a natural size photograph of a leaf fragment, which measures 3·3 inches long by 2·7 inches in the broadest part. Although the leaf is too fragmentary to indicate a true nature of its shape, base, apex, margins, etc., it is very well preserved as regards venation and nature of the primary and secondary veins; and by a comparison with a living leaf of *Populus ciliata* reproduced in Pl. VI, Fig. 14, it seems that the fossil leaf had probably a broadly ovate outline with a rounded or cordate base and an acute apex.

The venation is pinnate and reticulate. A fairly prominent midrib runs in the lamina thinning out in the upper part, and gives off 4–5 laterals which are almost half as thick, on either side, in a pinnate manner at acute angles. The basal lateral on the righthand side is profusely branched and gives off as many as 5–6 branches, one of which is seen to give off a branch of the third order; the upper laterals are branched only once or twice (Pl. VII, Fig. 15). Tertiaries form large rectangular meshes or cross-ties, which are preserved at some places in the fragment. Meshes of finer reticulations are not well preserved.

Our fossil leaf is identical in all respects with modern leaves of *P. ciliata* Wall. (Pl. VI, Fig. 14), a common poplar of the Himalayas.

Number of specimens.—Ten.

Occurrence.—Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collections.—R. R. Stewart, 1935.

Registered No. of figured specimen.—N 68.

There is another leaf fragment from the same collection which shows a good deal of resemblance to living leaves of *P. ciliata*, though absolute identity with this species cannot be claimed on account of the badly preserved nature of the fragmentary material. It is also figured here. Plate VI, Fig. 13 is a natural size photograph of the fragment, which shows a midrib with two to three pairs of laterals arising from it in an alternate manner

at acute angles like modern leaves of *P. ciliata.* The basal pair of secondaries which is branching in the fossil like *P. ciliata* seems to arise at angles slightly different from those in the latter species, and this may, to some extent, be accounted for a slight variation but assuming that the fossil leaf must have completely rotted in water before fossilisation, it may not be ridiculous to suggest that the present position of the laterals in the fossil is probably due to slight displacement of these from their original position and such an assumption is supported by an actual experiment performed in the laboratory on living leaves of *P. ciliata,* which on rotting became so soft and pulpy that the laterals lost their original distended position and in some cases even got broken from the midrib. But all this in the absence of a clear positive evidence is not sufficient to warrant accuracy in assigning the fossil to the modern species.

Number of specimens.—One.

Occurrence.—Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collector.—R. R. Stewart, 1936.

Registered No. of figured specimens.—N 100.

(2) *Populus nigra L.* var. *fastigiata* Desf.

(Plate VII, Figs. 16, 18)

Plate VII, Fig. 16 is a natural size photograph of a leaf fragment, which seems to have been triangular to sub-orbicular, or broadly ovate in outline with a feebly cuneiform base. A small part of the petiole measuring about ·4 inch in length is also preserved in one specimen.

The venation is pinnate and reticulate with a pseudo-palmate base. Two strong secondaries arise from basal part of the midrib a little above the base and run straight towards the margins, which are broken from the corresponding points of their ending, thus their course further ahead in the lamina cannot be ascertained. A fairly stout midrib, which slightly undulates and runs in a zigzag manner in the lamina, gives off 3–4 secondaries, thinner than itself, on either side in a sub-opposite, or alternate manner at acute angles. The laterals give off a number of branches on the outer side, and the latter tend to form indistinct loops by uniting with one another (Pl. VII, Fig. 16). Tertiaries form large meshes of various shapes and sizes, which further break up into a poorly preserved network of smaller meshes of finer reticulations.

In size, shape, margins and all details of venation our fossil leaf is identical with living leaves of *P. nigra* Linn. var. *fastigiata* Desf. one of which is reproduced in Pl. VII, Fig. 18 for a comparison.

Number of specimens.—Ten.

Occurrence.—Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collector.—R. R. Stewart, 1935.

Registered No. of figured specimen.—N 172.

There is another leaf fragment (shown in Pl. VII, Fig. 17) from the same locality, which shows sufficient similarity in shape, size, base, and apex with living leaves of *P. nigra,* but the four pairs of lateral veins in the fossil arise at acute angles in an alternate manner and seem to be closer and of a slightly different nature than what we find in living leaves of *P. nigra,* with which they seem to be closely related but not identical. Quite a few such leaves have been found in Stewart's collection.

(3) *Populus* sp. A

(Plate VII, Figs. 19–20)

Plate III, Fig. 19 is a natural size photograph of a leaf fragment, which seems to differ from the other two species of *Populus* described in the present paper. It measures 2·2 inches long by about an inch in the middle but a greater part of one half of the leaf in breadth is entirely missing. Though base and apex are also broken, their shape can still be made out and the leaf seems to have had an ovate-oblong lamina, with rounded base, and acute apex. The margin also is greatly damaged.

The venation is pinnate reticulate with a prominent midrib, which runs in the lamina slightly thinning out in the apical part. It gives off, on either side, in an alternate manner 5–6 secondaries at acute angles. The latter are about half as thick, and run straight towards the damaged margin, a little below which it is not possible to trace their nature. The lower two laterals close to the margin distinctly give off, on their outer and lower side, one or two branches, which show a tendency to curve upwards probably to form loops. The latter are quite thin, and are slightly distinguished from tertiary ribs, which either form cross-ties, or large rectangular meshes (Pl. VII, Fig. 20). Finer reticulation is not well preserved in the fossil.

Our fossil leaf does not show much similarity to any living species of *Populus* represented in the modern flora of India; but the form of venation is highly suggestive of its having some resemblance to another fossil leaf

figured in Plate VII, Fig. 17, which again has as much similarity with living leaves of *P. nigra.*

Number of specimens.—One.

Occurrence.—Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collector.—R. R. Stewart, 1935.

Registered No. of figured specimen.—N 260.

(4) *Populus* sp. B

(Plate VIII, Figs. 21–23)

Plate IV, Figs. 21 and 22 are natural size photographs of two counterparts of a fossil leaf, which is so incomplete and fragmentary that it is not possible to give a clear and exact description of its shape, base or apex. However, the form of venation suggests that the fossil might have had an ovate lamina with a cordate or rounded base and acute apex, more or less similar to living leaves of the other two species of poplars described above. The margins, too, are probably entirely missing.

The venation is pinnate-reticulate with the upper laterals prominently arched. A thick, strong midrib, which seems to run straight in the lamina, gradually thins out in the upper part, and gives off on its either side, at almost right angles, 4–5 equally prominent secondaries, which are approximately half as thick as the midrib. They run straight for about half the way in the fragment and beyond curve upwards forming semi-circular arches with the midrib. The lower secondary, a part of which is missing, gives off on its outer and lower side 2–3 branches, which at their ends curve upwards and tend to form loops in one counterpart (brought out in the photograph Pl. VIII, Fig. 22). In between two secondaries there arises a weaker secondary, which soon breaks up and gets lost into a network of the tertiaries, which also form cross-ties clearly seen in a part of the leaf (Pl. VIII, Fig. 22) enlarged to five diameters in the photograph Pl. VIII, Fig. 23. The tertiary meshes enclose a net work of smaller meshes, which constitute a finer reticulation.

The fossil leaf fragments do not match, in all details of venation, with any modern poplar of the Himalayas, though its venation is awfully like the genus *Populus*, with which it is, therfore, generically determined. On account of the incomplete nature of the fragments in which unfortunately none of the base, apex, or margins are preserved, it seems all the more reasonable that the specific determination of this fragment should not be

attempted especially when they do not seem to match any living species of Kashmir, or the nearby regions in the Himalayas.

Number of specimens.—Two (counterparts).

Occurrence.—Ningal Nullah, at 9,000 ft. in the Pir Panjal Range, Kashmir.

Collector.—G. S. Puri, 1941.

Registered Nos. of figured specimens.—Pl. VIII, Fig. 21=Loc. I N 51; Pl. VIII, Figs. 22-23 = Loc. I N. 52.

General Modern Distribution of the Salicaceæ

The family Salicaceæ—with 180 modern species distributed in two genera *Salix* and *Populus*—is, at the present time, chiefly north temperate in its global distribution; some species flourish on high mountain regions in alpine climates, also in the Arctic zone, in very severe atmospheric conditions, as a result of which these plants are reduced to stunted bushes or prostrate shrubs. There are, however, a few tropical species also, which are fairly common in warmer parts of the world. It is best represented in Europe, also in America and occurs in Africa, Asia and spreads through Malay Archipelago into Australia.

General Modern Distribution of *Salix*

Of the two genera, *Salix* is more widely spread, occurring in the Old as well as the New World. Its major areas of distribution in the northern hemisphere are North-West Europe, Central Europe, Southern Europe, the Mediterranean, and the Caucasus through which it seems to have spread into the Himalayas. It is equally well distributed in Pacific North America.

In India, there are as many as 26 well-determined species, besides a few doubtful ones, which are not taken into account in this paper. They are inhabiting different altitudinal zones in the Himalayas, ascending by some species to the snow-line and descending by others to as low elevations as sea-level in plains, where they are usually met with along streams or banks of fresh-water lakes. The largest number of Indian species occur in the Sikkim Himalayas. Western Himalayas and Temperate Himalayas come next as regards the number of species. A few are represented in Western Tibet. Dwarf willows are not uncommon in the Alpine Himalayas. One species is wild in the Deccan and another is found in the Khasi Hills, Assam. Burma affords a single species growing along streams. Willows and poplars are absent from Ceylon.

Modern Distribution of the Fossil Species of *Salix*

The Karewa species, as we shall see presently, are now mainly confined to the Western or Temperate Himalayas and do not seem to have a wider distribution at the present time. *Salix Wallichiana*, a graceful Himalayan willow, grows wild, or cultivated in the Temperate Himalayas, fairly continuously from Kashmir to Bhutan up to an altitude of 9,000 ft., and occurs also in the Punjab plains usually cultivated at water mills and along streams. Further east, it has been recorded by Gamble (1902, p. 687) at an altitude of 9,000 ft. but I could not confirm this either from literature, or Herbarium material. From Kashmir westwards, it is distributed in Afghanistan and has been recorded from the Kurram Valley (alt. 10–12,000 ft.) and has been collected by Stewart from the Kagan Valley in Hazara and Changla-Gali in the Murree Hills, where it occupies moister localities on sheltered aspects of the mountains.

The Kashmir distribution of the species, with which we are specially concerned here, is both interesting and significant. The species seems to have a much wider distribution now than in the Pleistocene time as is evidenced by its occurrence in the Karewa deposits. At the present time the species is reported to occur commonly in the Jhelum Valley (alt. 6,000 ft.), Baramulla and vicinity, and in Kamraj Division (alt. 6,500 ft.). On the Main Himalayan slopes it is abundant at Sonamarg and Gilgit and has been collected from Pahlgam in the Sindh Valley. In the valley proper it is often gregarious in moister localities and occurs commonly associated with *Populus ciliata, Juglans regia, Ulmus Wallichiana, Celtis alpina, Fraxinus excelsior, Parrotia jacquemontiana, Populus nigra,* etc., and with *Cedrus Deodara* and *Pinus excelsa,* as its coniferous associates, up to an altitude of 7,000 ft. It may be pointed out that the prevailing climatic conditions at this altitude of the valley are temperate and a fair amount of available water and atmospheric moisture in sheltered places combine together to create at this altitude ideal conditions for the growth of willows and poplars together with other mesophytic species.

Outside Kashmir *Salix Wallichiana* occurs in Pangi, Kulu, the Simla Hills, Jaunsar, Mussoorie, etc., of course, in moister localities and according to a sheet bearing the number 13955 in the Forest Herbarium, Dehra Dun, it ascends to an elevation of 12,500 ft. in Garhwal and is recorded from Kumaon Hills and also from Bhutan.

The second Karewa species—*Salix elegans*—is usually a high-level species, met with in the Western Himalayas from Nepal to Murree Hills, between the altitudes of 6,000 and 11,000 ft.; westwards it occurs as far as Kagan and in the Kurram Valley.

In Kashmir, at the present day, it seems to be more widely spread than the former species, where it occurs both on the Main Himalayan slopes–collected from Sonamarg (alt. 10,000 ft.), Pahlgam, Sindh Valley Division and the northern slopes of the Pir Panjal Range at Khillanmarg (alt. 9–10,000 ft.). It is no less common at other places and is recorded from the Kishenganga Valley, Kishtwar, Gurez, the Jhelum Valley, Keran, Marwa Dachhan, Muzaffarabad, Ramban and Udhampur. In the Kashmir Valley proper between the altitudes of 6,000 and 7 or 8,000 ft. it is rather scanty, occupying very moist places, but it is more common in the next higher zone—white birch zone—where it occurs commonly, associated with a more or less pure strand of *Betula utilis*, and *Syringa Emodi*, with *Lonicera* sp., *Rhododendron campanulatum*, *Pyrus foliolosa*, etc., at the altitudes of 10,500 ft. to 12,000 ft.

Outside Kashmir, eastwards, it is recorded from Kulu (alt. 10,700 ft.) Chamba, the Pangi, Simla Hills, Jaunsar, Deoban, Jabberkhet, Dharmsala, Tehri Garhwal, Almora District, Western Nepal, Kunawar, etc.; and also from the Niti Pass at an altitude of 11,500 ft.

General Modern Distribution of *Populus*

The genus *Populus* is comparatively less widely distributed than *Salix*, occurring mainly in the north temperate zone of both the Old and the New World. Unlike *Salix* it does not seem to extend to the Arctic regions, nor it touches snow-line in India or elsewhere, and alpine climates are not suited to poplars. The largest number of species are represented in Eastern Asia and Atlantic North America. A few species, however, are also fairly abundant in Europe.

In India we have only 6 species—5 are indigenous to the country and one is cultivated. Of these, three are found in the North-Western Himalavas, and one in the Temperate Himalayas. The warmer climates of the Punjab plains are fairly congenial to *Populus euphratica* and a single species occurs in Bhutan. It may be interesting to observe that four out of the six Indian species extend into Western Tibet and are also distributed over geographically much wider areas, extending into some parts of Asia and Europe.

Modern Distribution of the Fossil Species of *Populus*

Of the two well-determined Karewa species—one is restricted at the present time solely to some parts of the Temperate Himalayas, while the other has a much wider distribution, occurring in a wild form in Western Asia and Central Europe. It also enjoys a fairly wide distribution in a cultivated state in some parts of the North-Western Himalayas, especially Kashmir, the Punjab plains and Western Tibet.

Populus ciliata grows at the present time in Temperate Himalayan regions, from Kashmir to Bhutan at altitudes ranging from 4,000 to 10,000 ft., though it is best represented above 7,000 ft. In the Western Himalayas according to Gamble (1902, p. 690) it is fairly abundant in mixed forests of *Quercus Ilex, Quercus dilatata, Cedrus Deodara* and *Pinus excelsa,* but its conifer associate in the Kagan Valley as reported by Parker (1918, p. 510) is *Abies Webbiana.* In the Kashmir Valley, it is best represented to-day at an elevation of about 7,000 ft. above the level of sea and grows in the association of *Salix Wallichiana,* the latter occupying more moister places, and *Morus serrata, Morus alba, Celtis alpina, Fraxinus excelsior, Ulmus Wallichiana, Platanus orientalis, Parrotia jacquemontiana, Populus nigra, Cedrus Deodara, Pinus excelsa,* etc., in sheltered places or under mesophytic conditions.

From Kashmir westwards, it extends in the Kagan Valley in Hazara and flourishes in the neighbouring regions in the Murree Hills occurring at Dunga-Gali in association with *Aesculus indica, Prunus cornuta, Juglans regia,* etc., in the mixed coniferous forests of *Pinus excelsa* and *Taxus baccata.*

Its Kashmir distribution, in which we are specially interested here, is fairly wide; in addition to the valley proper the species is recorded both from the southern slopes of the Main Himalayas and the northern slopes of the Pir Panjal at various places including Gurez, the Jhelum Valley, Kamraj, Keran, Kishtwar, Marwa Daccha, Ramban, Muzaffarabad, the Sindh Valley, Udhampur, Sonamarg, etc.

Populus ciliata is fairly gregarious outside Kashmir, too, and its occurrence as taken from authentic sheets in the herbaria may be mentioned eastwards from Kashmir in Monali and Nagni in the Kulu Valley, Kalatop at Dalhousie, Matiyana and Nagkanda in the Simla Hills, Bashahr, Mussorie and Dharmsala; and also in gregarious patches on the China slope at an altitude of 7,000 ft. in Naini Tal.

The second well-determined Karewa species, *Populus nigra* var. *fastigiata* popularly known as " the Lombardy poplar ", is not indigenous to our country and affords an excellent example of how a foreign plant has become completely naturalized to our climates. It is believed to have been introduced into India from somewhere in Western Asia where it grows wild. It also grows in Central Europe, at the present time.

In India it is cultivated in the North-Western Himalayas from Simla westwards and grows equally well in plain country at Rawalpindi. It is very common in Kashmir growing profusely here and there and occurs everywhere in the valley as a roadside tree, or often hewn down in hedges; on the surrounding mountains it occurs on the Kashmir slopes of both the

Main Himalayas and the Pir Panjal Range ascending there to the altitude of 10,000 ft. It extends well into Western Tibet, where again it is abundantly cultivated up to an altitude of 12,500 ft.

From Kashmir westwards, it is cultivated in the Kagan Valley, Hazara, Abbottabad, the Muree Hills, etc., and occurs in Baluchistan; in Afghanistan. J. L. Stewart (1874, p. 472) states that Griffith observed *P. nigra* growing in a wild state at Shekhabad near Kabul at an altitude of 7,500 ft.—a fact of great importance, which may throw some light on the origin of this species in Kashmir. The relative ease and rapidity with which the Lombardy poplar seems to have acclimatised itself to Kashmir conditions is amazing; one often finds in spring old completely cut off stumps of many trees sprouting into shoots, and sprouting of dried branches lying here and there or half buried in soil for purposes of tying cattle is not an unusual sight for those familiar with the country.

Its common associates in the Kashmir Valley are *Salix Wallichiana, Populus ciliata, Morus serrata, Morus alba, Juglans regia, Pyrus communis Prunus cornuta,* etc., etc.

Outside Kashmir, eastwards it is recorded from Pangi, Chamba and Bashahr as a cultivated tree.

The unmistakable occurrence of a good many fossil leaves of *Populus nigra* var. *fastigiata* in the fossiliferous beds of the Pleistocene Age at Ningal Nullah and the fact that this species at the present time grows so profusely in Kashmir may force one to cast a little doubt on the belief of some botanists that the species is of a recent introduction to Kashmir. If the testimony of fossil plants be accepted as trustworthy towards the solution of this question then it is clear that the theory of a recent introduction of this species to Kashmir does not hold good in the face of the above-said facts and one may tentatively suggest that the existence of this species in Kashmir, at least near Ningal Nullah, is as old as the Pleistocene times.

May be that *Populus nigra* var. *fastigiata* had become extinct in Kashmir at the close of Pleistocene times and it had now spread all over the valley like a wild fire in a few years time after its recent introduction to this region on account of the fact that it had found most congenial conditions for its rapid growth; but this statement is as difficult to prove as to disprove at the present time and we may have to wait till we get further evidence to prove our contention. It may be worth while to mention that the Lombardy poplar has not flowered in Kashmir and is propagated solely by vegetative methods.*

* Since this was written the author (1945) has sent a paper to *Indian Forrester* in which he has proved that the Lombardy poplar is indigenous to the Kashmir Valley.

General Fossil Distribution of the Salicaceæ

A good many fossil species of the Salicaceæ based mostly on leaves, or catkins, closely allied to, or identical with modern species, have been discovered from the Northern Hemisphere. They occur in great abundance, among the rocks ranging in age from the Pleistocene to the Cretaceous. We find innumerable references to the fossil Salicaceæ in the treatises dealing with the Tertiary Floras of America and Europe, also in Asia, though the Tertiary Floras of this vast continent are still very imperfectly known. A detailed account of the fossil history of the Salicaceæ, which deserves a separate paper to itself, is outside the scope of the present work and may be reserved for a later occasion.

In spite of the availability of a vast literature dealing with the Salicaceæ, the question of its palæogeographic distribution and its original home still remains undecided because there exists a great confusion on the validity of certain fossil species. The earlier students of Tertiary Floras being unaware of the ecological methods in plant determinations either wrongly identified fossil species, or gave widely unrelated generic names to undoubtedly Salicaceous leaves. Such unfortunate examples of erroneous interpretations are by no means confined to the Salicaceæ. Similar mistakes have been made in other Dicotyledenous families included in the Tertiary Floras of America and Europe. To repeat with Graham (1939, p. 124), " Much of the published information regarding the geographic and temporal distribution of the various plant groups in past ages is unquestionably incorrect, and many evolutionary trends have been obscured by these same faulty records ".

Although the palæogeographical distribution of the Salicaceæ is still imperfectly known there is evidence to show, as Rendle (1938, p. 9) believes, that the family had a wider distribution in Tertiary times than it has to-day. Fossil remains of *Salix*, mostly leaves and catkins, have been discovered in Tertiary localities within the Arctic Zone and most northern regions of America and Europe, showing that the family once extended, probably, to more northernly regions in the past than it does at present. Similarly, fossil leaves belonging to *Populus* also have been discovered in the same formations in the Arctic Zone, although poplars are unknown in these regions at the present time.

The Karewa species (excepting *Salix* sp. A and *Salix* sp. C) were discovered at the Ningal Nullah locality, the fossil flora of which is remarkably different from that of the other fossiliferous localities in having an assemblage of purely temperate species.

Discussion

The fossil occurrence at Ningal Nullah of a few well-determined species of the Salicaceæ, which are fairly abundant in the modern flora of the Kashmir Valley to-day, may indicate that this part of the valley, at least, had at one time in the past enjoyed a cooler climate essentially similar to what we find at the present time along the northern slopes of the Pir Panjal Range and the south-eastern end of the Kashmir Valley at an altitude of about 7,000 ft. above sea-level. This conclusion, which in this paper is primarily based on the evidence of fossil Salicaceæ, is nevertheless supported by the discovery, at the same locality, of several other species. A comparison between the past and present distribution of these species, whether considered individually, or collectively, as a whole, seems to indicate in unmistakable terms that cooler climates had once existed in Ningal Nullah in the past also, and that the climate of this part of the valley has probably remained unchanged since the Pleistocene.

To mention a few of the other species collected from Ningal Nullah we find that *Juglans regia, Acer Cæsium, Pyrus Malus, Aesculus indica, Rhamnus purpurea, Prunus cornuta,* etc., are the past as well as the present associates of the fossil Salicaceæ in the forest flora of Kashmir. A flora composed of these fossil species, at the present time, grows along the northern slopes of the Pir Panjal Range and flourishes between the altitudes of 7,000 and 8,000 ft., though some of the species may extend to even higher altitudes of 10,000 ft. or more. The fossil species have been discovered from the beds, which are now lying at an elevation of 9,000 ft. Comparing the past and present altitudinal ranges of the flora we find that there is hardly a significant difference between the two. One might very well infer from the terrestrial element alone that the fossil flora was probably deposited at the same altitudes at which it is now discovered. But the occurrence in the same beds in an heterogenous mixture with terrestrial plants of a leaf fragment of *Nelumbo nucifera* (Puri, 1944) and a few parallel-veined bits of plants comparable to living leaves of more than one species of *Sparganium* and *Typha* (see Puri, 1941, pp. 7-8) alters the evidence considerably. We have to take into account the modern distribution of these species, which are common aquatic plants of the Kashmir lakes and have never been seen growing in this region at altitudes higher than 5,200 ft.—the present elevation of the valley.

In the light of the above facts it seems there is a little room for doubt in concluding that the fossil plants discovered from Ningal Nullah were deposited at lower elevations—about 5,200 ft. above sea-level—and that the

beds had since been uplifted and elevated to these higher altitudes by the Pleistocene Himalayan uplift, which affected the whole of the Karewa Series. The occurrence in the fossil beds of some of the terrestrial plants represented among the fossil flora, which are now growing at the altitude of 8,000 ft., or higher, may be explained by assuming that they must probably have been washed down by streams flowing through the forest into the Karewa Lake.

The Significance of Temperate Species in the Fossil Flora

The Karewa floras hitherto discovered at other localities are principally composed of such fossil species, which, at the present time, are common trees of sub-tropical or tropical rain forests of the outer Himalayan ranges. The occurrence at Ningal Nullah of the Salicaceæ and other fossil species (in a more or less pure assemblage), which are now inhabiting cooler and moister aspects of the Kashmir Valley, is highly significant. As already stated, it indicates that this part of the valley during the Pleistocene times had probably experienced a temperate climate not very different from those that now prevail near the fossiliferous outcrops.

It is interesting to find that at Laredura (another outcrop at a distance of about five miles from Ningal Nullah) at an altitude of 6,000 ft. above sea-level there occurs a different type of flora composed mainly of three dominant species of oaks, namely, *Quercus semecarpifolia, Q. dilatata,* and *Q. Ilex* and many other less important species, *e.g., Carpinus viminea, C. faginea, Fraxinus xanthoxyloides, Olea glandulifera, Engelhardtia Colebrookeana,* etc. These species are unrepresented in the modern flora of the Kashmir Valley or the northern slopes of the Pir Panjal Range; but they instead, form an important floristic formation in tropical montane forests. While the dominant species of this flora are conspicuous by their absence from the Kashmir Valley to-day, there are quite a few other fossil species, namely, *Aesculus indica, Rosa Webbiana, Ulmus Wallichiana, U. lævigata, Betula utilis, Desmodium tiliæfolium,* etc., which are still growing in these regions. This flora, as we shall see presently, is intermediate between the Ningal Nullah and Liddarmarg floras.

The fossil flora discovered from a third locality (Lidddarmarg) is still more different in species from that of Ningal Nullah and indicates a climate of a pronounced sub-tropical nature. Here among the dominant plants of *Quercus incana, Q. glauca* and some members of the Lauraceæ, namely, *Machilus Duthiei, M. odoratissima, Litsea lanuginosa,* we find other less common species, *e.g., Buxus papillosa, B. Wallichiana, Acer oblongum, Ficus Cunia, Phoebe lanceolata,* etc. All these species are absent from the

valley at the present time but on the other hand they constitute an important element of the sub-tropical rain forests of the outer Himalayas.

It is interesting to note that the three fossiliferous localities, which have yielded three different fossil floras—the first indicating a cooler climate and the other two seemingly tropical and sub-tropical in their climatic aspects—are assigned the same geological age by De Terra (see De Terra and Paterson, 1939, pl. 55), who also stated that the floras preserved at these localities were deposited during the First Interglacial Period in the Himalayas.

With a view to understand the past climatic conditions of these regions let us reconstruct some of the important topographical features of the valley during the Pleistocene. The fossil plants provide ample evidence to show that the Pir Panjal Range, which now extends along the southern border of the valley, forming a formidable barrier to summer monsoon winds, was, at a time when oak forests were flourishing in these regions represented by a low ridge, over which the tropical and sub-tropical elements present in the fossil floras had extended into those parts from where they are absent to-day. The subsequent uplift of this range brought about important changes in the climate and vegetation of the valley, which was now cut off from the water-laden monsoon winds and thus furnished congenial conditions for the growth of temperate species in place of tropical plants, most of which became extinct from the northern slopes of the Pir Panjal Range and are, therefore, unrepresented in the modern flora of this region (Puri, 1943, pp. 128–31).

The discovery of temperate species among the deposits of the same age exposed at Ningal Nullah which indicates a cooler climate, is really interesting and it may raise a pertinent question to explain how during the same phase of the Pleistocene, the three fossil floras, discovered from three different localities (now situated within a radius of about 30 to 35 miles), were growing on the northern slopes of the Pir Panjal Range contemporaneously at adjacent places? If we presume that the three floras belong to the same Interglacial Period the difference in the specific composition of the three floras may be attributed to other causes, some of which may be local variations in topography, soil, proximity of the lake, climate, etc. But it may also be possible to suggest that they were growing in different Interglacial Periods.

In view of the fact that a part of the work on this collection is still to be completed it may seem premature to offer comments in favour of any probability at this stage, therefore, a detailed discussion of this question is reserved for a later occasion.

SUMMARY

1. Nine fossil species of the Salicaceæ are described from leaf impressions among the material collected partly by R. R. Stewart in 1936 and partly by the author in 1940 from a few spots from the Ningal Nullah locality lying near Botapathri at an elevation of 9,000 ft. above sea-level ; the strata are exposed on a wide grassy meadow on the right bank of Ningal Nullah stream. The fossiliferous beds belong to the Lower Karewa Series and are Pleistocene in Age.

2. Of the nine species described in this paper there are four, namely, *Salix Wallichiana, S. denticulata, Populus ciliata* and *P. nigra* var. *fastigiata* which are fully determined, the remaining five incompletely determined species include three belonging to *Salix,* which could not be identified specifically on account of the fragmentary nature of the plant material; and two distinct species of *Populus,* that do not seem to resemble any modern Himalayan species.

3. All the fully determined species are represented in the modern flora of the Kashmir Valley, and *Salix Wallichiana* occurs at the present time in temperate parts of the Himalayas from Kurram Valley (alt. 10–12,000 ft.) through Kashmir to as far east as Garhwal (alt. 12,500 ft.), Nepal and Bhutan. It also occurs in moist places in the inner dry valleys of Pangi and Kagan. *Salix denticulata* is commonly found in the north-western Himalayas extending eastwards also like the first species, from the Kurram Valley to Western Nepal. *Populus ciliata,* the common Himalayan poplar, also grows in temperate regions between the altitudes of 4,000 and 10,000 ft., from the Kagan Valley through Kashmir to Bhutan. *Populus nigra,* var. *fastigiata* which is wild in Kabul is believed to be cultivated at the present time in the Western Himalayas up to the altitude of 12,500 ft.

4. A comparison of the past and present distribution of the well determined fossil species, which are still growing in the Kashmir Valley near the fossiliferous region at the present time indicates that this part of the valley, at least, at one time during the Pleistocene had probably enjoyed a temperate climate essentially similar to modern climate of the valley, and this would have allowed the growth of temperate species like *Juglans regia, Rhamnus purpurea, Prunus cornuta, Pyrus Malus, Acer Cæsium,* etc., all of which are the present as well as the past associates of the Salicaceæ.

5. Though the terrestrial element of this flora as a whole does not indicate in clear terms that the uplift of the fossiliferous beds had taken place after their deposition, the presence among them of an aquatic element represented by a leaf fragment of *Nelumbo nucifera,* and some parallel-veined

leaf fragments comparable to modern species of *Sparganium* and *Typha*, shows beyond doubt that the beds had been uplifted by the Pleistocene Himalayan Orogenies.

6. While discussing the significance of temperate species in this flora it is pointed out that the other two floras discovered at Laredura and Liddarmarg indicate a prevalence in the valley, during the Pleistocene, of tropical or sub-tropical climate opposed to a cooler climate indicated by the Ningal Nullah species.

7. The causes of the occurrence during the Pleistocene of three different floras on the northern slopes of the Pir Panjal Range of that time contemporaneously at three localities are also hinted at but a fuller discussion of this question is reserved for a later occasion.

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Explanation of Plates

The figures are from untouched photographs and specimens are preserved in the Botany Museum, University of Lucknow. They are of natural size where otherwise stated.

Plate V. *Salix Wallichiana* Anders.

Fig. 1. Fossil leaf impression . R. R. Stewart collection, 1935, Registered No. N 132, Ningal Nullah (alt. 9,000 ft.), Pir Panjal Range, Kashmir Valley, *Karewa Series* (*Pleistocene*).
Fig. 2. A part of the leaf enlarged to show details of venetion. × *Ca.* 5.
Fig. 3. Living leaf for comparison with the fossil.

Salix sp. A

Fig. 4. Fossil leaf impression. R. R. Stewart collection, 1935, Registered No. N 203, Ningal Nullah.
Fig. 5. A part of the leaf (Fig. 4) enlarged to show details of venation. × *Ca.* 5.

Salix elegans Wall.

Fig. 6. Fossil leaf impression. R. R. Stewart collection, 1935, Registered No. N 20, Ningal Nullah.
Fig. 7. A part of the leaf (Fig. 6) enlarged to show details of venation. × *Ca.* 5.

Salix sp. C

Fig. 8. Fossil leaf impression. G. S. Puri collection, 1940, Registered No. Loc. 1 N 10, Ningal Nullah.

Plate VI. *Salix elegans* Wall.

Fig. 9. Fossil leaf impression. G. S. Puri collection, 1939, Registered No. L 150, Laredura (The piece of twig lying near the base of the leaf does not seem to belong to it).

Salix sp. B

Fig. 10. Fossil leaf impression folded on itself at the middle. G. S. Puri collection, 1939, Registered No. L 600, Laredura (alt. 6,000 ft., Pir Panjal Range), Kashmir Valley, *Karewa Series* (*Pleistocene*).
Fig. 11. A part of the leaf (Fig. 10) enlarged to show details of venation. × *Ca.* 5.
Fig. 12. A part of the leaf (Pl. V, Fig. 8) enlarged to show details of venation. × *Ca.* 5.

Populus ciliata (?)

Fig. 13. Fossil leaf impression. R. R. Stewart collection, 1935, Registered No. N 100, Ningal Nullah.

Populus ciliata Wall.

Fig. 14. Modern leaf partly rotted in water for comparison with the fossil leaves (Pl. VI, Fig. 13 and Pl. VI', Fig. 15).

Plate VII. *Populus ciliata* Wall.

Fig. 15. Fossil leaf impression. G. S. Puri collection, 1941, Registered No. Loc. I N 15, Ningal Nullah.

Populus nigra Linn. var. *fastigiata* Desf.

Fig. 16. Fossil leaf impression. R. R. Stewart collection, 1935, Registered No. N 172, Ningal Nullah.
Fig. 17. Fossil leaf impression. R. R. Stewart collection, 1935, Registered No. N 50, Ningal Nullah.
Fig. 18. Modern leaf partly rotted for comparison with the fossils (Figs. 16, 17).

Populus sp. A

Fig. 19. Fossil leaf impression. G. S. Puri collection, 1941, Registered No. Loc. I N 45, Ningal Nullah.
Fig. 20. A part of the fossil leaf (Fig. 19) enlarged to show details of venation. × *Ca.* 5.

Plate VIII. *Populus* sp. B

Fig. 21. Fossil leaf impression. R. R. Stewart collection, 1935, Registered No. N 150, Ningal Nullah.
Fig. 22. Counter-part of fossil leaf (Fig. 21) broken and slightly smaller than N 150.
Fig. 23. A part of the leaf (Fig. 22) enlarged to show details of venation. × *Ca.* 5.

3

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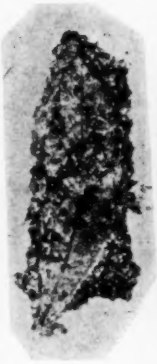

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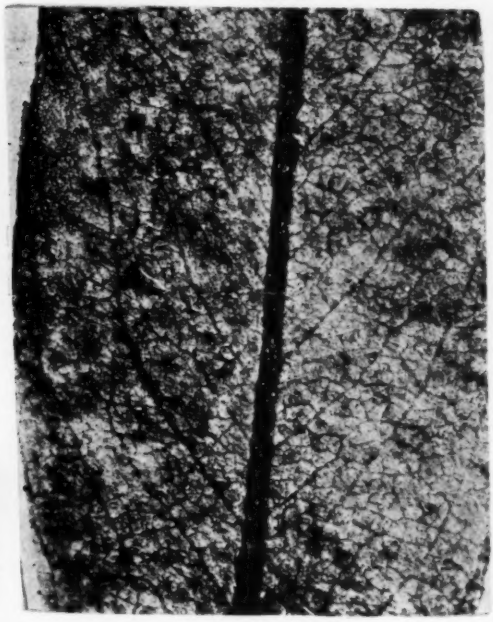
2×Ca. 5



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7×Ca. 5

5×Ca. 5

11×Ca. 5

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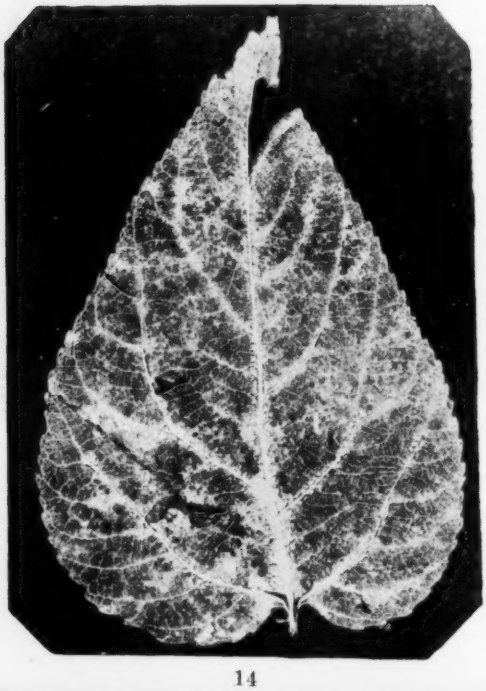

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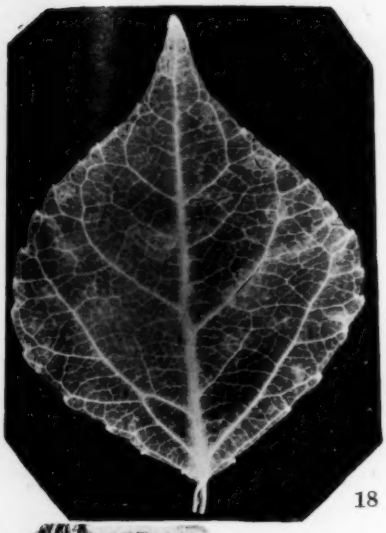
12×Ca. 5



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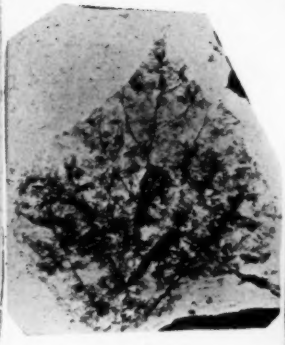

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20×Ca. 5

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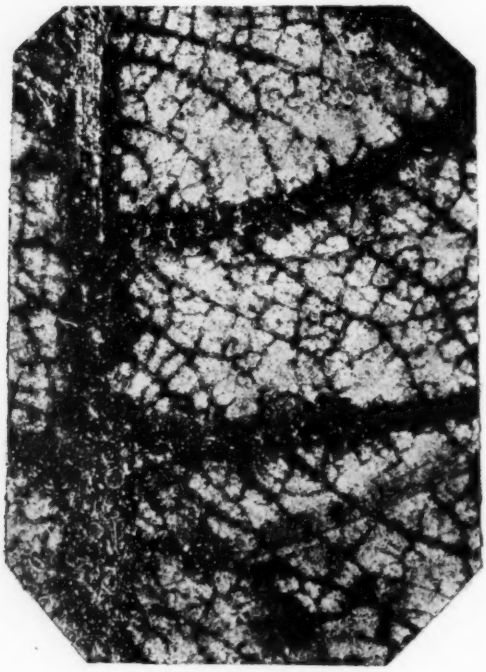

23 ×Ca. 5

THE CORPUS LUTEUM IN *CHILOSCYLLIUM GRISEUM* (MULL. AND HENLE)

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Received March 24, 1945

(Communicated by Prof. R. Gopala Aiyar, F.A.SC.)

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INTRODUCTION

OUR knowledge of the structure and development of the corpus luteum in elasmobranch fishes is practically limited to the work of Giacomini (1896) on *Myliobatus bovina* and of Wallace (1904) on the formation of the corpus luteum in *Spinax niger*, both of which are viviparous forms. Very recently the author (1943) has published an account of the histology and development of the corpus luteum in an ovo-viviparous form, *Rhinobatus granulatus*. The present paper on the corpus luteum in *Chiloscyllium griseum* from the Indian coast, is the first investigation of its kind dealing with the formation of the corpus luteum in an oviparous elasmobranch and has been chosen as likely to lead to interesting results.

MATERIAL AND METHODS

The material for this study was obtained from two gravid females one captured in April and the other in August, 1940. The ovary, unlike that of *Rhinobatus granulatus*, is single and median in position. It contains usually ova of various sizes, the larger ones being bluish green while the smaller are light yellow in colour. The first specimen had two egg capsules in the same uterus. Bouin's and Carnoy's fluids were used as fixatives and

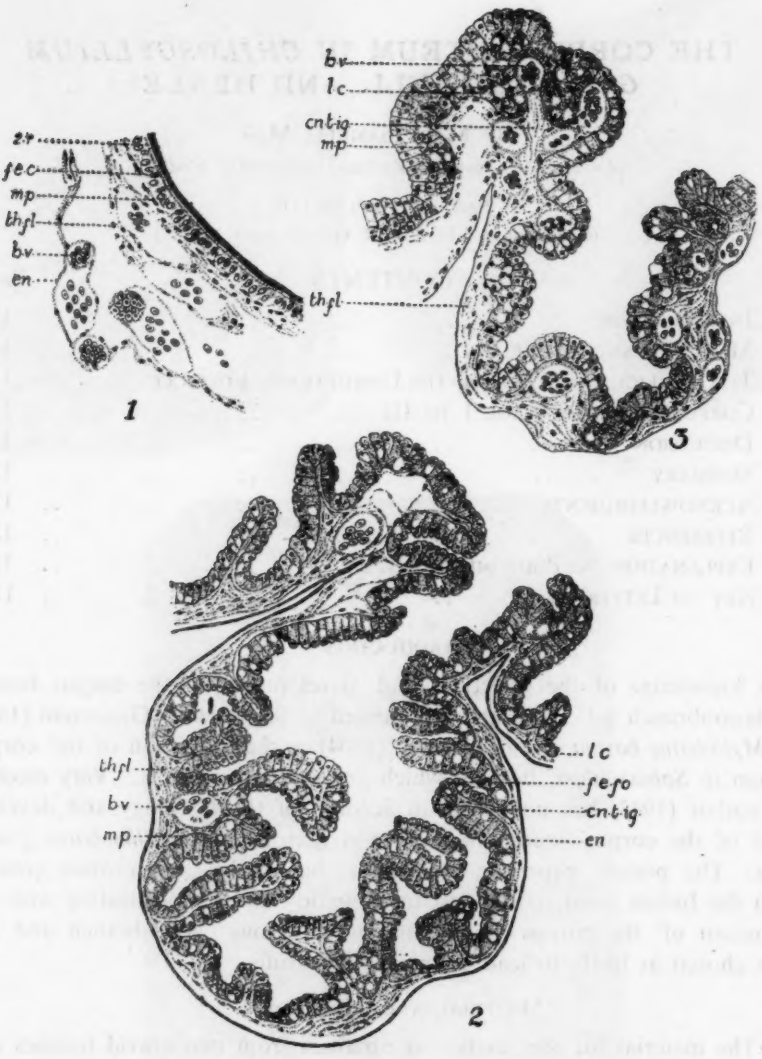

Fig. 1. Transverse section of the ripe ovum showing the follicular wall. ×120.

Fig. 2. Transverse section of the corpus luteum (Stage I), showing the highly folded wall and the ingrowth of connective tissue. ×80.

Fig. 3. Transverse section of the corpus luteum (Stage II), showing the luteal cells and the invasion of blood vessels in association with the connective tissue theca. ×80.

both gave good results. Sections were stained with iron hæmatoxylin and as counterstains Eosin and Van Gieson were employed.

The Follicular Wall of the Unruptured Follicle

As in all elasmobranchs the egg membranes surrounding the ovum consist of an outer homogeneous vitelline membrane closely pressed against an inner zona radiata. The latter is radially striated, is distinct and stains deeply (Ph.m. 1 and Fig. 1).

The follicular epithelium.—The follicular epithelium, in contrast to that of *Rhinobatus granulatus*, is a single layer of uniform columnar cells (Ph.m. 1 and Fig. 1). In this respect *Chiloscyllium griseum* shows similarity to *Spinax niger* (Wallace, 1904), *Mustelus lævis* (Giacomini, 1896) and many other fishes. The cylindrical cells lie with their long axes perpendicular to the surface of the egg and possess prominent large oval nuclei radially disposed, with a well marked nuclear reticulam carrying deeply staining, small chromatin granules. Occasionally the nucleoli can be distinguished. The cytoplasm is homogeneous and stains lightly. The follicular cells are provided with fine processes which seem to be attached to the egg membrane. Wallace (1904) describes similar delicate protoplasmic processes for the corresponding cells of *Spinax niger* 'the distal ends of which are fixed in the egg membrane'. A nutritive function has been attributed to the cells of the follicular epithelium and the fine processes are probably the structures by which the nutriment is conveyed to the egg.

The membrana propria.—The follicular epithelium is covered on the outside by a fine homogeneous membrane—the membrana propria (Ph.m. 1 and Fig. 1). This uniform layer consists of a single layer of elongated protoplasmic cells with deeply staining elongated nuclei. This membrane probably represents a specialised layer of the theca folliculi.

The theca folliculi.—The whole egg is contained within a vascular connective tissue sheath—the theca folliculi (Ph.m. 1 and Fig. 1). It is a distinct and well marked but is a narrow layer, consisting of protoplasmic connective tissue cells with ovoid or elongated nuclei filled with fine chromatin granules and indistinct cell limits. These cells are readily distinguishable from those of the follicular epithelial cells. A few fibrous strands of connective tissue run in between the cells. The cells and fibres are disposed parallel to the surface of the egg. In contrast to the condition in *R. granulatus* the connective tissue theca is thinner and is without differentiation into theca interna and theca externa. Blood vessels of varying sizes pass through this layer.

Corpus Luteum

Stage I

The corpora lutea are small sac-shaped structures measuring about 12 mm. The small size is probably the result of contraction which has taken place consequent on the rupture of the follicle and escape of the ova. The ruptured opening closes almost immediately after the extrusion of the egg.

Photomicrograph 2 and Fig. 2 represent a portion of a transverse section of the corpus luteum at the earliest stage studied. It is a hollow structure with a conspicuous lumen in the centre containing a lightly staining fluid and surrounded by the follicular wall. The histology of the wall of the corpus luteum is as follows. It consists of the same three layers which invest the ripe egg but these layers have undergone histological differentiation (Ph.m. 3 and Fig. 2). In this stage the follicular epithelium is thrown into very prominent folds by the force of contraction caused by the rupture of the follicle and subsequent escape of the egg (Ph.m. 2 and Fig. 2). Unlike what happens in *R. granulatus* the follicular epithelium does not separate from the connective tissue sheath with the result that the theca is carried inwards along with the follicular epithelial folds. The cells of the follicular epithelium have undergone remarkable changes and are totally unlike those constituting the unruptured follicle both in size and cytological details. There is to begin with a great increase in the size of the cells. In this hypertrophy of the cells a similarity to the higher vertebrates is noticed. The cells are at first elongated and columnar having distinct cell membranes with their nuclei placed at their free ends (Ph.m. 3 and Fig. 2). During the transformation of the follicular epithelial cells into luteal elements the nucleus assumes a distinctly vesicular shape in contrast to the oval nuclei of the follicular epithelial cells of the unruptured follicle. With the assumption of the luteal condition a distinct deeply staining nucleolus and chromatin reticulum becomes a feature of the nucleus. The cytoplasm is dense and finely granular, staining intensely with iron hæmatoxylin. Intra-cellular vacuoles are seen in the cytoplasm of some of these cells. Inter-cellular spaces are also observed in the follicular epithelium. The single layered condition of the follicular epithelium is retained, except in certain regions where it presents a many layered appearance. No multiplication of cells by karyokinesis has, however, been noticed. Hence the enormous hypertrophy of the follicular epithelial cells together with the general contraction and folding of the follicular wall and the pushing in of some of the cells of this layer must account for the multilayered condition that is observed in certain regions. It may be stated in this

connection that in the earliest stage described in *Spinax niger* by Wallace (1904) the follicular epithelium is thick and composed of many layers of cells. It should be considered as more advanced than the one described in *Chiloscyllium* where the follicle for the most part is single layered.

The membrana propria is still seen as a thin but distinct layer investing the follicular epithelium. It is drawn inwards along with the follicular epithelial folds (Ph.m. 3 and Fig. 2).

The theca folliculi.—In most of the higher vertebrates the theca surrounding the follicular layer of the corpus luteum shows specialisation into the theca interna and theca externa to a varying extent. In *Chiloscyllium* the connective tissue theca does not show any differentiation into two layers at any stage. It is represented as a narrow layer consisting of connective tissue cells and fibres. The nucleus of the thecal cells are small and oval presenting a nucleolus and chromatin reticulam. The boundaries of the thecal cells are indistinct and could be made out only occasionally. This layer has undergone a certain amount of stretching on account of the hypertrophy of the follicular epithelial cells. However, the theca shows an advance on the unruptured follicle as strands of cells and fiibres can be traced as radial ingrowths into the follicular epithelium. There is a considerable increase in the vascularisation of the layer, especially at the periphery, where some of the thecal cells and fibres can be seen enveloping the wall of the blood vessels. The endothelium consisting of elongated cells with flattened nuclei retains its previous condition as a definite layer surrounding the corpus luteum.

Stage II

The fully formed corpus luteum presents a striking resemblance to the advanced stage described by Wallace in *Spinax niger* (Ph.m. 4 and Fig. 3), but is totally different from the fully mature gland in *R. granulatus*. As compared with the mammals, reptiles and certain viviparous elasmobranchs the corpus luteum here is very simple and small. There is also an appreciable reduction in the central lumen owing to the further contraction of the follicle and the further ingrowth of the connective tissue theca pushing the hypertrophied follicular epithelial layer towards the central lumen. The latter contains a lightly staining coagulam in which are suspended a few darkly staining globules.

The corpus luteum in most of the animals so far studied is a hollow structure in the early stages which subsequently becomes filled in by the ingrowth of the hypertrophied luteal cells together with connective tissue

elements, so that the fully developed corpus luteum is a solid body closely packed with luteal cells. It is interesting that in this form the hollow condition persists with the central lumen greatly reduced even in the fully developed condition. A hollow corpus luteum has been described by O'Donoghue (1916) in a marsupial *Phascolarctos cinereus* which that author considers to be unique among mammals. It is noteworthy that a similar condition should be present in two groups of animals so widely separated in their zoological position. No great significance can be attached to it.

The structure of the follicular wall is illustrated in photomicrograph 4 and Fig. 3. The follicular epithelium has become many layered to a greater extent though the single layered condition is also seen in certain regions. No evidence of cell division has so far been observed and hence it may be assumed that this many layered condition is attained by the further hypertrophy of the luteal cells resulting in some of the cells being pushed inwards leading to an overlapping of cells. The luteal tissue shows spherical to columnar cells with distinct cell limits. These cells are exclusively derived from the follicular epithelium. The nuclei of the cells have attained a larger size and each typically contains a large intensely staining irregular nucleolus and numerous fine chromatin granules. The cytoplasm in most of the cells is delicate and vacuolated. There is some reduction in the staining capacity of the cells. Careful examination shows indications of commencing degeneration evidenced by the vacuolisation of the cells. Intra-cellular vacuoles are more abundant and show an increase in size in some of the cells. Occasionally, the vacuoles are so large that they occupy the whole cell with the nucleus pushed to the periphery. But some of the cells however show a normal appearance having vesicular nuclei.

The theca has become more pronounced than in the previous stage (Ph.m. 4 and Fig. 3). The nuclei of the theca cells have become larger. The nucleoli are only occasionally distinct. The most remarkable feature is the extensive development of radial ingrowths at various points, of connective tissue fibres and cells. This phenomenon recalls the condition in the corpus luteum of mammals and reptiles. There is further vascularisation of the theca and large blood vessels can be observed at various points round the theca folliculi. Another advance on the earlier stage is the ingrowth of blood vessels in association with the radial connective tissue ingrowths which lie in close contact with the follicular epithelium, though not actually among the luteal cells. This ingrowth of blood vessels in the luteal tissue is a characteristic feature of mammals as well as of certain reptiles.

It may be recalled that Wallace (1904) in dealing with the highly advanced corpus luteum in *Spinax niger* describes degeneration and depicts the presence of degenerating cells both in the follicular epithelium and in the theca and also free blood corpuscles in the lumen. The stage described here for *Chiloscyllium* closely corresponds to that mentioned by Wallace but the degeneration is not so advanced, as only slight vacuolation is observed in the luteal cells and no free blood corpuscles or degenerating cells are found in the lumen.

Stage III

The last stage studied represents a fairly advanced state of degeneration (Ph.m. 5). The reduction in size has been continued and the radial ingrowths have disappeared. The central lumen still persists though it is very much reduced and is filled with groups of degenerating luteal cells and blood corpuscles (Ph.m. 6). These cells in the internal lumen resemble the cells of the follicular epithelium and are evidently detached from it. They have small, deeply staining nuclei and the cytoplasm persists as a thin layer round the dense mass of nuclear material. Free nuclei without the cytoplasmic covering are also seen. Cell degeneration has spread throughout the luteal tissue as is revealed by the presence of shrunken cells with ill-defined cell boundaries containing collapsed nuclei. Further evidence of degeneration is offered by the occurrence of large vacuolar spaces surrounding the small pycnotic nuclei in which neither the nucleoli nor the chromatin reticulum could be seen owing to the clumping together of chromatin granules. Free blood corpuscles have penetrated in between the degenerating follicular cells and this invasion is widespread along the whole inner layer of the follicular epithelium.

The difference between the theca and the follicular epithelium has become very indistinct (Ph.m. 6). The cells of the theca show advanced signs of degeneration and are disposed in masses where cell boundaries are indistinguishable. The nuclei show evident signs of regression. They are very much smaller and stain intensely. The chromatin granules are very indistinct and are clumped together into a mass. This early degeneration of the corpus luteum stands in contrast to the long functional activity of the gland in *R. granulatus*.

Discussion

The corpus luteum of *Chiloscyllium*, an oviparous form, is of interest as it shows some points of resemblance to that of viviparous elasmobranchs and higher vertebrates in certain fundamental features while revealing

peculiarities of its own in other respects. Marshall (1922), in his review of the history of the formation of the corpus luteum in different groups of vertebrates, states that the lower vertebrates which show hypertrophy of the follicular epithelial cells of the ruptured follicle, are all viviparous. Cunningham and Smart (1934) draw the general conclusion that the true corpus luteum is "the consequence and result of the internal development of the fertilized ovum in oviduct or uterus, or in the cavity of the ovary, and that in oviparous forms the ruptured follicle at once begins to undergo reduction and absorption". Investigations of Hett (1924) on *Lacerta agilis*, of Weekes (1934) on *Amphibolurus muricatus* and the present study on *Chiloscyllium griseum*, on the other hand, show that the hypertrophy of the follicular epithelium resulting in the formation of a corpus luteum is apparently more general and extends to oviparous forms as well. The corpus luteum of *Chiloscyllium* exhibits close similarity to that of *Spinax niger*, an essentially viviparous form, described and figured by Wallace (1904). There is similarity in the general mode of development of the corpus luteum in the elasmobranchs and in the higher vertebrates. The formation of a corpus luteum in a way similar to that found in the higher vertebrates is probably true for all elasmobranchs. But the development of the ruptured Graffian follicle into a solid glandular body fundamentally similar to the gland found in mammals judging from our present knowledge is more in evidence in viviparous and ovo-viviparous elasmobranchs than in oviparous forms. This is clear when the corpus luteum in *Myliobatus* (Giacomini, 1896) and *Rhinobatus* (Samuel, 1943) is compared with that in *Chiloscyllium* where it is a simple hollow structure. The latter, however, is of special interest as it exhibits an approach to the mammalian condition in the hypertrophy of the follicular epithelial cells, and the ingrowth of connective tissue theca into the follicular epithelium along with the blood vessels in association with the thecal ingrowths; these taken together cannot be without significance.

The luteal cells arise exclusively from the follicular epithelial cells as a result of their hypertrophy and histological differentiation. In *Chiloscyllium* the distinction between the cells of the theca and the follicular epithelium is clear throughout. At no time do the cells of the theca approximate in size and structure to the cells of the follicular epithelium and there is no indication that the former are modified to form luteal cells. This stands in sharp contrast to the complicated development of the corpus luteum in *R. granulatus* where a fair proportion of the theca interna cells transform into luteal cells similar to those formed from the follicular epithelium. It has been noted that the structure of the fully developed luteal cells shows

considerable difference from that of *R. granulatus*, particularly in regard to the changes that take place in the cytoplasm, despite the close zoological relationship.

Regarding the early degeneration of the corpus luteum in oviparous forms, Weekes (1934) states that in the oviparous lizard *Amphibolurus muricatus* the corpus luteum disappears after a short intra-ovarian existence, soon after the laying of the egg, while in the viviparous lizard the gland has a long duration of functional activity. The shorter period of activity of the corpus luteum in oviparous forms is probably true also of the elasmobranchs as seen from a comparison of its functional life in *R. granulatus* and in *Chiloscyllium griseum*. The fact that the eggs are retained only for a comparatively short time (Aiyar and Nalini, 1938) in the uterus may explain the rapid degeneration of the corpus luteum in *Chiloscyllium*.

Summary

1. The fully formed corpus luteum in *Chiloscyllium griseum* is a simple hollow structure. The hypertrophied follicular epithelium, the membrana propria, the theca and the endothelium constitute the wall of the corpus luteum.

2. The luteal cells are derived exclusively from the follicular epithelial cells.

3. The theca does not show any differentiation into theca interna and theca externa. It exhibits an approach to the mammalian condition in regard to the ingrowth of connective tissue theca into the follicular epithelium and the invasion of blood vessels along with the thecal ingrowths.

4. Degeneration sets in early and the corpus luteum has only a short duration of functional activity.

Acknowledgments

I take this opportunity for acknowledging my indebtedness to Professor R. Gopala Aiyar, for his guidance and valuable criticism. I am also indebted to the Syndicate of the University of Madras for awarding me a Research Studentship during the tenure of which this work was completed.

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* Not referred to in the original.

Explanation of Photomicrographs

Photomicrograph 1. Transverse section of the ovum showing the follicular wall.

„ 2. Transverse section of the corpus luteum, Stage I, showing the folded follicular wall of the corpus luteum.

„ 3. A section of a portion of the wall of the corpus luteum, stage I, showing the hypertrophied follicular epithelial cells and the ingrowth of connective tissue.

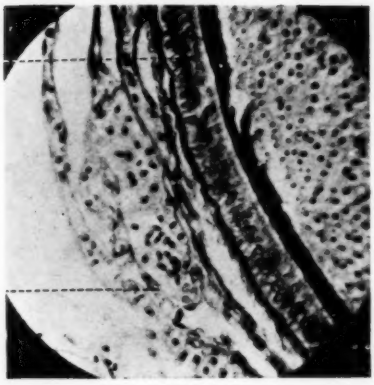
„ 4. Transverse section of the corpus luteum, Stage II; a portion showing the multilayered condition and the ingrowth of blood vessels along with the connective tissue theca.

„ 5. Transverse section of the degenerating corpus luteum, Stage III.

„ 6. A portion of the wall of the corpus luteum, Stage III, magnified to show the degenerating luteal cells.

Key to Lettering

b.co. ..	Blood corpuscles.	*f.e.fo.* ..	Follicular epithelial fold.
b.v. ..	Blood vessel.	*l.c.* ..	Luteal cells.
cn.t.ig. ..	Connective tissue ingrowths.	*mp.* ..	Membrana propria.
d.l.c. ..	Degenerating luteal cells.	*th.fl.* ..	Theca folliculi.
en. ..	Endothelium.	*z.r.* ..	Zona radiata.
f.e.c. ..	Follicular epithelial cells.		



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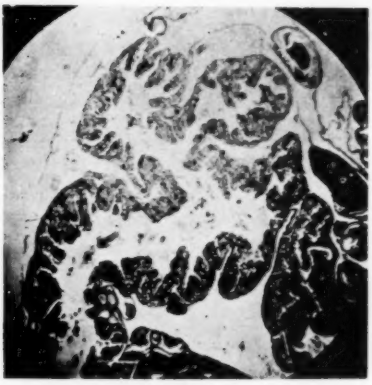

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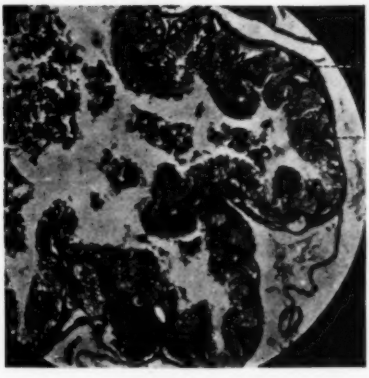

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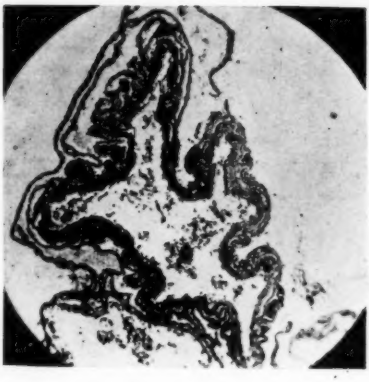

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THE MODE OF ACTION OF DRUGS ON UNSTRIATED MUSCLE AND THE NATURE OF INHIBITION

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Received April 4, 1945

The action of potassium appears to depend upon a difference in its concentration within and without the fibres, both in unstriated muscle (Singh, 1939) and in striated muscle (Wright, 1942). The mode of action of drugs appears to be different. According to Clark, they attach themselves to sensitive patches on the cell surface, and according to Straub, they act by a virtue of a difference in concentration of the ions on two sides of the muscle membrane, during the process of permeation.

In unstriated muscle, most of the contractions and inhibitions fall into two groups. In this paper, the properties of contractions and inhibitions produced by certain drugs are described, and the mode of their action elucidated.

Results

Action of Acetylcholine

In the electrolyte-free medium :

In this medium the frog stomach becomes hyperexcitable as regards the excitability which produces the spontaneous contractions. The spontaneous contractions may be three times larger in the half tonic sucrose solution than in the saline; this proves that the excitability of the frog stomach is not depressed. The action of acetylcholine in this medium has been very difficult to ascertain. Out of 12 stomachs in six it produced no contraction. This suggests that the contraction produced by acetylcholine is due to sensitisation to ions in the saline, as it is not produced in the absence of such ions, though the excitability of the muscle is otherwise normal. In the remaining six muscles, acetylcholine produced a contraction, but it was smaller than in the saline, and indistinguishable from the sopntaneous contractions; therefore when acetylcholine was added, it was difficult to be certain whether the contraction was due to the drug, or it was one of spontaneous contractions. As it was however a constant phenomenon, I am inclined to believe that it was a contraction produced by the drug.

The effect of the electrolyte-free medium therefore shows that acetylcholine produces two kinds of contractions, one by ions outside the fibres, and the other by ions inside.

Frog stomach.—In this muscle acetylcholine produces two kinds of contractions. One kind of contraction resembles the potassium contraction, in that (i) it is increased if the chloride of the saline is replaced by iodide, which increases the excitability to potassium and ammonium, but depresses that to alternating current. Small concentrations of iodide (0·00560 M NaI) increases the excitability to potassium as well as alternating current by antagonising the inhibitory action of sodium. Greater increase in concentration of iodide, decreases that to alternating current but increases that to potassium owing to its potentiating the action of the latter. If increased beyond 0·056 M, then the excitability to potassium also is depressed; but this action is variable, the concentration which increases the excitability may vary from 0·01 M till the whole of the sodium chloride is replaced by iodide. (ii) It is decreased by increase in osmotic pressure to 1·4 times normal. This contraction is increased by eserine (1 in 10^5). The other contraction has the properties similar to those of contraction produced by alternating current. (1) The optimum concentration of calcium is the same. (2) It is increased by increase in osmotic pressure of the saline to 1·4 times normal at pH 8, sodium chloride being used in increasing the tonicity of the saline. (3) The optimum temperature is the same. (4) It is depressed by iodide. (5) It is decreased by small concentration of eserine (1 in 10^5).

The above results are in agreement with the findings in skeletal muscle, that acetylcholine is capable of causing contracture, as well as twitch. Acetylcholine therefore mobilises potassium within the fibres. From this, it would appear that acetylcholine does produce a contraction in the electrolyte-free medium.

A curious fact was noticed, that the stomach and heart muscle from certain frogs were absolutely refractory to acetylcholine (1 in 10^8 to 1 in 10^6), even after eserine (1 in 10^7 to 1 in 10^4). Like *Mytilus* muscle, they could withstand high concentration of eserine. It is therefore difficult to conceive how acetylcholine could act as transmitter, especially when muscle responded normally to potassium, and alternating current; the latter is known to produce a propagated response. This supports electrical rather than chemical transmission.

Dog stomach.—In the dog stomach the criterion distinguishing the two excitabilities is the concentration of calcium and hydrogen ions.

Here also acetylcholine produces two kinds of contractions. In one kind the optimum concentration of calcium is the same as that for the contraction produced by alternating current (0·002 M $CaCl_2$). In the other, the calcium required varies from 0·007 to 0·03 M $CaCl_2$, so that it belongs to the potassium group. Acetylcholine, therefore, mobilises potassium within the fibres, and increases the sensitivity to ions outside.

Rabbit gut.—In this muscle, acetylcholine produces similar result as in dog stomach.

Guinea pig uterus.—The contraction is favoured by excess of calcium.

Mytilus *muscle.*—Acetylcholine causes a tonic contraction, decreases the excitability to alternating current and increases that to potassium. It differs from the latter, that replacement of the sodium of the saline with lithium decreases the contraction. If excess of potassium has not depressed the excitability, then the contraction produced by acetylcholine is potentiated. This shows two actions of acetylcholine. (1) It produces contraction belonging to the potassium group. (2) Its action depends on the action of ions out side the fibres.

Many *Mytilus* muscles were found absolutely inexcitable to acetylcholine, in fact to chemical stimulation in general. Therefore it is difficult to understand how acetylcholine could be a transmitter of the nervous impulse to the muscle. The muscle responded to alternating current, which produced a propagated response. It is therefore reasonable to suppose that transmission was electrical.

In these muscles, treatment with eserine (1 in 10^6) restored the response not only to acetylcholine, but also to alternating current, potassium. This shows that the action of eserine was a general one on excitability, rather a specific one on any choline esterase. Tone was diminished, when the excitability otherwise was increased; eserine caused relaxation, while acetylcholine caused contraction. Large concentrations of eserine diminished the response to alternating current, but increased that to potassium.

Adrenaline contraction :

Mytilus *muscle.*—The action depends upon the sensitivity of the muscle. In muscles which are sensitive, adrenaline (1 in 10^5) causes contraction, increases the excitability to potassium, stretch, barium; diminishes that to alternating current, and direct current, increases the off-contracture and viscosity, diminishes the rate of relaxation and of stretch. Smaller concentrations increase the excitability to electric current and diminish tone.

In insensitive muscles, adrenaline (1 in 10^5) increases the excitability to electric current, diminishes viscosity and tone. Caffeine (saturated solution in *Mytilus* saline) abolishes the insensitivity. Caffeine in such muscle increases the excitability to alternating current, twitch, potassium.

These results indicate, that the adrenaline contraction belongs to the potassium group, that is produced by the ions outside the fibres.

Frog stomach.—In the frog stomach adrenaline rarely causes a contraction. The contraction is followed by inhibition. The contraction occurs in the electrolyte-free medium, thus showing that adrenaline mobilises potassium inside the fibres.

Such a phenomenon is also produced in the *Mytilus* muscle in the absence of calcium, or if the chloride is replaced by anions such as iodide, in rabbit gut in excess of potassium by alternating current. The initial contraction is followed by inhibition. With diminution of excitability the initial contraction disappears so that inhibition only remains (Fig. 1). As this secondary contraction is adaptation, inhibition and adaptation are therefore identical. Inhibition is therefore a secondary process; it is an excitatory process overcome by adaptation. When a substance produces excitation, adaptation is a concurrent phenomenon, and if the latter proceeds as fast as or faster than the former, then only inhibition would be produced. More intense the excitatory process, the greater will be the accompanying inhibitory process, and with greater concentration of the drug, both the inhibitory and excitatory processes may be increased in such a way that the former predominates. In small concentrations, the drug will produce contraction and with large concentrations inhibition.

It is probably for this reason that many processes affect excitation and inhibition identically, because in the latter, the excitation which is masked, is really the primary process. A substance may thus produce excitation or inhibition. The inhibitory process being the result of excitatory process, rises and subsides more slowly than the latter, with the result that contraction in dog stomach by alternating current, potassium, acetylcholine, vagus stimulation (Narayana and Singh, 1944) is followed by inhibition. If the excitatory and the inhibitory processes balance each other, then on addition of the drug, there will be no significant change, but on withdrawl of the drug, an inhibition would be produced (Singh, 1942).

Another phenomenon frequently met with in unstriated muscle is that in which a substance first produces inhibition, and then contraction. Such action is produced by ammonium, adrenaline, thiocyanate in dog stomach. In this instance, the excitatory process catches up with the inhibitory pro-

cess. On withdrawal, a contraction or inhibition might result. The former will be produced if adaptation takes place to the inhibitory state, by increase in the excitatory state, and the latter when such an adaptation is very slow. Thus on addition of a substance, there may be (1) contraction; (ii) inhibition; (iii) contraction followed by inhibition; (iv) inhibition followed by contraction; (v) no visible effect. On withdrawal, there may be (i) contraction; (ii) inhibition; (iii) or no visible response.

Dog stomach.—Adrenaline may cause contraction or inhibition. The contraction resembles that produced by acetylcholine, in that the concentration of calcium required is about 0·007 to 0·01 M $CaCl_2$. If however adrenaline and acetylcholine are added together, the total tension is not greater, but less than that produced by acetylcholine alone. This is an example of occlusion in unstriated muscle. It is difficult to explain. It means that the excitatory process set up by adrenaline is antagonistic to that produced by acetylcholine. On withdrawal of adrenaline, the excitability to acetylcholine is enhanced, or withdrawal contraction may be produced. Repeated withdrawal of adrenaline may cause the muscle to enter into tonic contraction.

Eserine (1 in 10^5) increases the excitability not only to acetylcholine, but also to adrenaline, and alternating current. This shows that the action of eserine could not be one simply combining with the choline esterase.

Ephedrine (1 in 10^5) may also cause contraction of the dog stomach and potentiate the response to adrenaline, but after the muscle has adapted to ephedrine, the action of adrenaline is antagonised. This shows that the potentiation of adrenaline by ephedrine is due to the summation, so that it acts by other means besides preventing the destruction of adrenaline.

Adrenaline inhibition :

Gunea pig uterus.—Concentration of adrenaline used was 1 in 10^7. Potassium normally present in the saline (0·00616 M KCl), though itself producing marked inhibition (Singh, 1942), antagonises the inhibitory action of adrenaline (Fig. 2 A, B). This is an example of occlusion in unstriated muscle. Excess of calcium or potassium not sufficient to produce a tonic contraction, potentiates the inhibition. Excess of potassium, which produces tonic contraction antagonises the inhibition.

Calcium, normally present in the saline (0·00206 M CaCl) potentiates the adrenaline inhibition. In the absense of calcium adrenaline may fail to produce inhibition, and may even produce contraction (Fig. 2, D). This resembles the action on heart muscle, wherein inhibition is lessened in the

absence of calcium (Gunn, 1944). Excess of calcium favours inhibition. Effect of sodium chloride is variable. If the sodium chloride content of the saline is decreased upto 20% normal, the inhibition by adrenaline increases just as the inhibition by ammonium in the frog stomach. After that the inhibitory effect of adrenaline decreases.

In isotonic solution of sucrose, the muscle passes into a tonic state, and adrenaline fails to have any effect at all. Introduction of calcium and potassium fails to have any effect. Only on the reintroduction of sodium chloride, that the inhibitory effect of adrenaline returns. Sodium chloride is therefore necessary both for certain contractions and inhibitions in unstriated muscle.

In the electrolyte-free medium (Singh, 1944 *a*) adrenaline fails to have any effect, but the introduction of saline produces inhibition, showing that the muscle has not lost the power of inhibition and that external ions are necessary for the action of adrenaline.

The action of hydrogen ions is variable. They potentiate as well as antagonise adrenaline inhibition. If the reaction is changed from acid (pH 6·5) to alkaline (pH 8), the adrenaline inhibition at first increases and then decreases.

Direct current antagonises as well as potentiates adrenaline inhibition. This is probably due to the fact that it produces both kinds of inhibitions. Its antagonistic action is also shown by the fact, that at pH 8, when maximum inhibition has developed by direct current and adrenaline together, the stoppage of the current produces further inhibition (Fig. 2, C). Exactly opposite happens at pH 6·5, because at this pH, the synergistic inhibition is more easily produced by the current.

Ammonium antagonises the adrenaline inhibition, though by itself it may produce inhibition.

The effect of osmotic pressure is most remarkable. Moderate increase in osmotic pressure (1·1 times normal) may increase the inhibitory action of adrenaline (Dale, 1913), but further increase causes a marked decrease; the inhibitory effect of adrenaline may be abolished or converted into an excitatory one.

Acetylcholine antagonises the inhibition.

Optimum temperature for adrenaline inhibition is 30°C; at 37°C, it may be absent.

Rabbit gut.—Potassium antagonises adrenaline inhibition; the effect of calcium and hydrogen ions is uncertain. Increase in osmotic pressure decreases the inhibition.

Frog stomach.—The reactions are similar to those in the guinea pig uterus. Eserine increases the inhibition.

In the electrolyte-free medium, adrenaline loses its power of producing inhibition. The inhibitory action previously referred to (Singh, 1944 *a*) was on the spontaneous contractions by heavy doses (1 in 10^4). If the muscle is thoroughly washed, then adrenaline loses its effect.

Action of Pilocarpine

This was studied on the rabbit gut and dog stomach. The action was same as that of acetylcholine. Pilocarpine (1 in 10^5) in dog stomach may produce inhibition. Withdrawal of pilocarpine may cause contraction or augmentation of the spontaneous contractions.

Action of Pituitarin

This was studied on the guinea pig uterus. It causes a tonic contraction, which is improved by calcium and decreased by increase in osmotic pressure.

Discussion

Drugs which act in minute concentrations, either produce contractions or inhibitions which are a class by themselves, or they produce contractions belonging to both groups. Certain effects of these drugs suggest that they sensitise the muscle to ions outside (Singh, 1943). Their action is antagonised by increase in osmotic pressure, that is, increase in the concentrations of ions inside the fibres.

The concentration of the drug is too minute *per se* to have any effect on the ionic ratio on the two sides of the muscle membrane. The action must therefore be produced by ions outside the fibres, that are present in significant amount. It is probable that these drugs affect the same structure, acted upon by calcium that is the muscle membrane. They probably produce a change in permeability (Singh, 1944 *b*).

Adrenaline loses its inhibitory effect in the electrolyte-free medium, and in the absence of sodium chloride. This suggests that its action is produced by some constituent of the sodium chloride. Its action is antagonised by potassium, electric current and hydrogen ions. These are the ions which antagonise the action of sodium chloride, again suggesting that adrenaline sensitises the muscle to sodium or chloride.

It is possible that sodium produces contraction or inhibition, depending upon the reaction, and that chloride produce contraction. Adrenaline may produce inhibition by sensitising to sodium and contraction by sensitising

to chloride. When adrenaline and acetylcholine are together (they individually producing contraction, then their action will be synergistic if they sensitise to chloride, and antagonistic if one sensitises to sodium and the other to chloride.

As the action of adrenaline increases in the presence of calcium, it is possible that these drugs act on the muscle membrane by changing the state of calcium. If free calcium in the membrane is suppressed, then a contraction would occur, and if calcium is liberated then inhibition would occur. This view is supported by the fact that though stimulating ions and tone are antagonistic to inhibition, yet a moderate increase favours inhibition. An increase in the excitatory state leads to an increase in the inhibitory state. With moderate increase, the excitatory state just exceeds the inhibitory state, so that on the addition of an inhibitory substance, the balance is upset in favour of inhibition. With great increase, the excitatory state greatly exceeds the inhibitory state, and the combined action of the inhibitory substance and the inhibitory state is less than the excitatory state.

A substance may thus affect inhibition by its inhibitory action *per se*, and by its action on the tone, and these two actions may be antagonistic. Thus the substance may produce different effects in different circumstances. Eserine increases tone; this may result in increase or decrease of adrenaline inhibition.

SIMILARITIES BETWEEN THE RESPONSES OF UNSTRAITED MUSCLE AND THOSE OF NERVOUS SYSTEM—

The responses of the unstriated muscle resemble those of nerve structure, such as the sensory nerve endings and the neurone. Many of the responses which are regarded as peculiar to the nervous system are found in the unstriated muscle.

Properties of the peripheral sense organs and those of unstriated muscle.— In response to a constant stimulus, they respond with a rhythmic succession of nerve impulses. If a constant stimulus in the form of constant current is applied to a nerve fibre, an impulse is set up when the current is made. Plain muscle presents similar phenomenon when stimulated with alternating current; it may respond by (i) continuous tension, (ii) rhythmic contraction, or (iii) only by initial contraction.

Properties of the neurone and unstriated muscle.—In the neurone there are two states, the central excitatory state and the central inhibitory state; the latter outlasts the former. These two states are also found in unstriated muscle, the inhibitory state outlasting the excitatory.

Occlusion.—This is well seen in the unstriated muscle. Thus in the dog stomach, both adrenaline and acetylcholine cause contraction, but when added together the total tension is less than that produced by acetylcholine alone, may be, in fact equal to difference intead of the sum of two.

Subliminal fringe.—In *Mytilus* muscle, both 0·1 M KCl, and replacement of the chloride of the saline by iodide might cause contraction. In insensitive muscle, both may fail to produce a response, but when potassium is added after iodide, a response is obtained. If they both cause contraction in the first instance then their combined action produces greater tension, than the sum of the two when acting singly.

After dischage.—A single stimulus may set up a repetitive discharge. Thus on cessation of alternating current, the muscle may go on contracting rhythmically for some time. In summer *Mytilus* muscles, treated with *Mytilus* saline containing caffeine and adrenaline, there may be rhythmic after-discharge after a single stretch.

Rebound.—The tonic withdrawal contractions are the same phenomenon; it is due to the fact that adaptation takes place to inhibition by increase in the excitatory state.

Recruitment and after-discharge.—Normally the frog stomach when stimulated with alternating current may contract only during the passage of the current, but when cooled, the tension rises more slowly, and continues to rise some time after cessation of the stimulus.

Inhibition may also show recruitment. Thus on stimulating the guinea-pig-uterus with direct current (14 volts), the inhibition may continue after the cessation of the current.

Fractionation.—Normally when the muscle is stimulated with alternating current, it responds with only a fraction of the tension that it is capable of responding under suitable conditions.

Stimulation by hydrogen ions.—The respiratory centre is stimulated by hydrogen ions. Plain muscle may do likewise, but is much less sensitive. Other stimulus may act identically on both. Magnesium depresses tone, as well as nervous activity. Bromides may produce inhibition in the central nervous system as well as plain muscle. Strychnine makes *Mytilus* muscle hyperirritable to potassium.

Summary

(1) Both adrenaline and acetylcholine produce contraction of unstriated muscle in the electrolyte-free medium, suggesting that their action is due to mobilisation of ions within the fibres.

(2) They also produce contractions or inhibitions resembling those produced by ions outside the fibres; this suggests that they may also act by sensitising the muscle to ions outside.

(3) Acetylcholine produces two kinds of contractions in the unstriated muscle.

(4) Inhibition is really an excitatory process, masked by adaptation.

(5) Adrenaline inhibition is antagonised by potassium, ammonium, electric current, hydrogen ions, and increase in osmotic pressure; it is potentiated by calcium, hydrogen ions, and also by electric current. Adrenaline thus produces two kinds of inhibitions.

(6) Eserine acts by means other than combining with choline-esterase.

(7) Ephedrine potentiates adrenaline also by a process of summation.

(8) Drugs produce a contraction, which is a class by itself.

(9) The optimum temperature for adrenaline inhibition is 30°.

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Explanation of Plates

Fig. 1. *Mytilus* muscle: The genesis of the inhibition. Stimulation with alternating current for 5 minutes

A. In *Mytilus* saline.

B. Chloride of the saline replaced with bromide.

C. Chloride of the saline replaced with nitrate. Tone is neutralised during adaptation to current.

D. Chloride replaced with iodide. Inhibition occurs during passage of the current. This inhibition is easily seen to be identical with adaptation.

Fig. 2. Guinea pig uterus

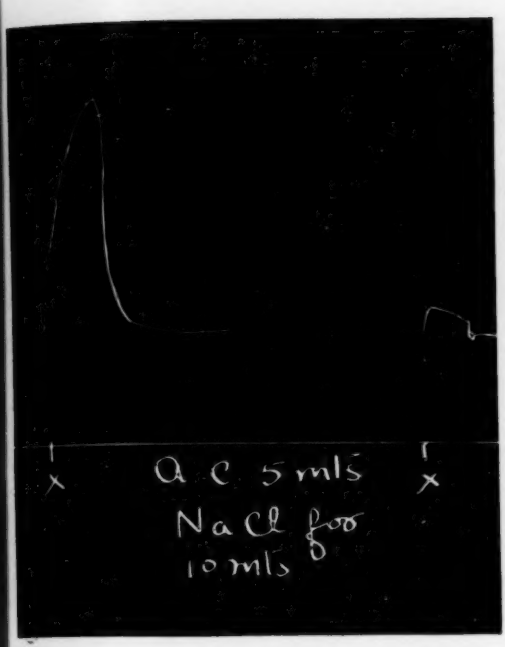
A. Inhibition produced by adrenaline (1 in 10^7) in the presence (+K) and absence of potassium (−K).

B. Same as A.

C. Inhibition produced by D.C. (12 volts) and adrenaline from X to Y; at Y the current is stopped.

D. Effect of calcium on adrenaline inhibition.

E. Effect of temperature on adrenaline inhibition.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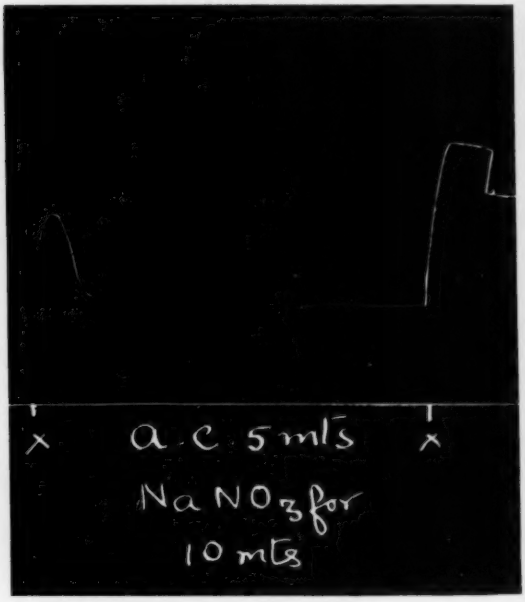
Na Cl for
10 mls

A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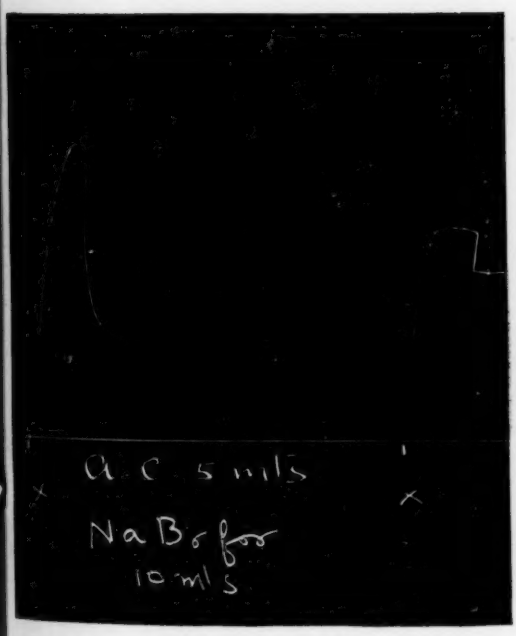
C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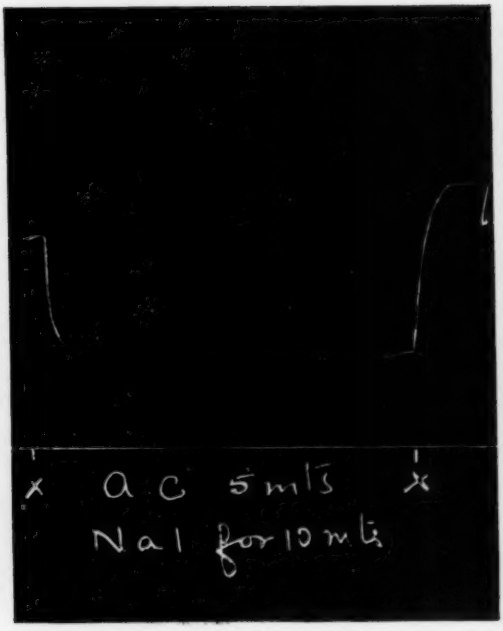
Na Br for
10 mls

B


a c 5 mls
Na I for 10 mls

D

FIG. 1

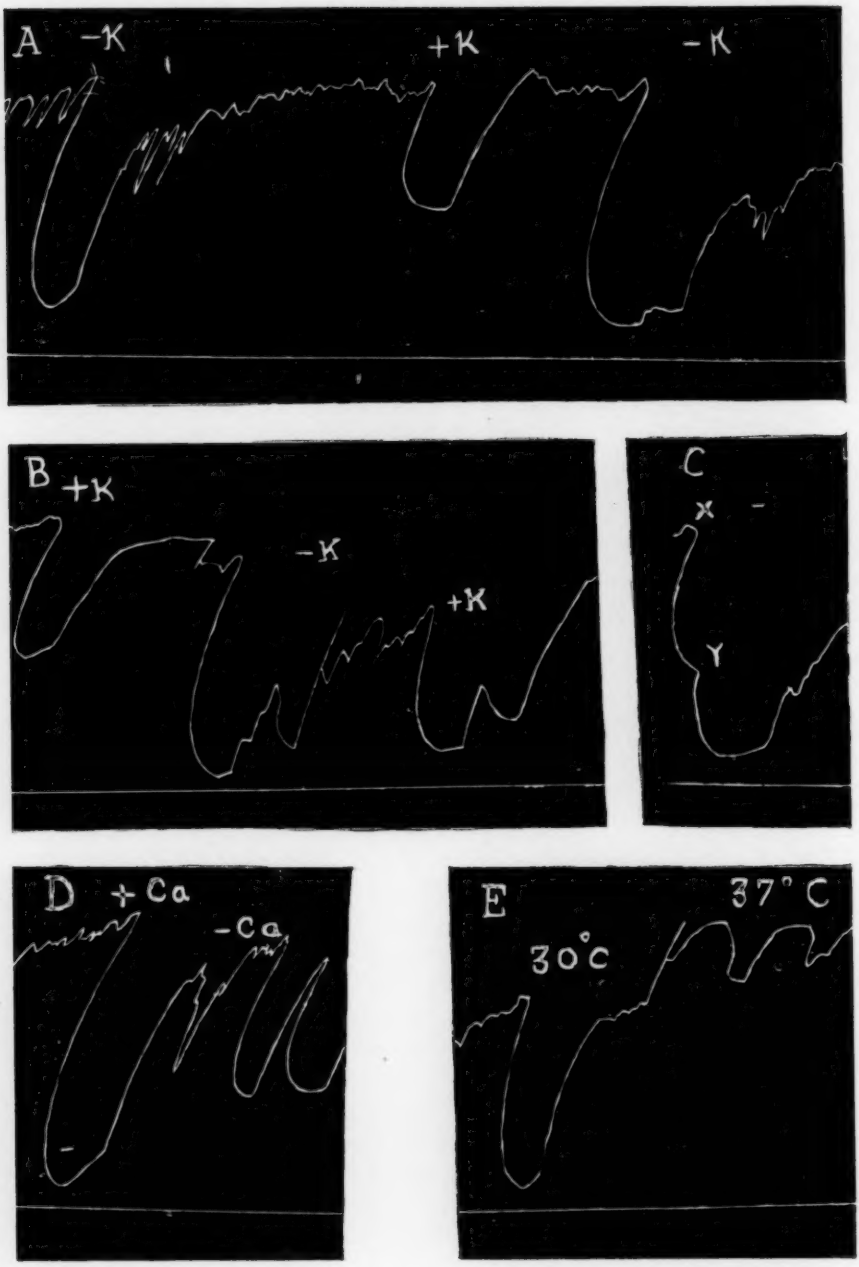


FIG. 2

PHYSICO-CHEMICAL ASPECTS OF THE APPLICATION OF MERCURY TOWARDS PRESERVATION OF STORED FOOD GRAINS

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Received March 14, 1945

(Communicated by Prof. D. D. Karve, F.A.SC.)

The use of mercury for protecting stored food grains is an age-old custom in several parts of India. Dr. K. K. Kanan first brought the insecticidal property of mercury in connection with storage of grains, to the notice of the entomologists in the third Entomological Conference in India.[1] Since then various experiments have been made with mercury for preservation of wheat, rice and other grains from spoilage by insects. It is now well established that this property of mercury is due to the small amount of vapour which it emits at atmospheric temperatures. The mercury vapour sterilises the insect eggs and breaks the life-cycle of the insects feeding on the grain. It has been further observed[2] that not only the eggs but also small larvæ of the insects are killed when mercury amalgams or colloidal mercury dispersions (dusts) are used for preservation of grain. There is an agreement among all previous workers that it is the mercury vapour that is responsible for checking insect growth. This means that mercury is acting as a fumigant and not as a contact or stomach poison. Naturally, therefore, the emission of mercury vapour from the different preparations of mercury is the chief factor of their efficiency as preservation of grain during storage.

At ordinary temperatures metallic mercury is a liquid with nonwetting properties. Evaporation of a liquid, in the space where diffusion of its vapour is rapid, depends upon its vapour pressure at the temperature at which the vaporisation is taking place, and the surface of the liquid exposed for evaporation. On account of its high surface tension mercury has a tendency to coagulate in droplets, which entails a great decrease in its evaporating surface, as compared with that which it otherwise would have had, if mercury had been a liquid with wetting properties and low surface tension. Hence there is a very slow formation of vapour from mercury in its ordinary state. A larger surface of evaporation than the one in the liquid state of mercury, rapid diffusion of mercury vapour around the grain to be preserved, minimum loss of mercury vapour away from the space of grain storage

[1] *Proc. 3rd Ent. Meeting, Pusa*, 1919, 761–62.

[2] Dole, *J. Uni. Bom.*, March, 1943, **11**, Part V, 117.

and the availability of all the amount of mercury in the preparation which is to be used for preserving grain, are some of the conditions essential for use of mercury for the preservation of stored food grains in an effective and efficient manner.

On account of the liquid state of mercury at ordinary temperatures it requires some vehicle to support it during the course of its application as a preservative in the storage of grain. Since the efficiency of mercury depends upon the evolution of its vapour and its diffusion, use of such a vehicle should facilitate vaporisation and diffusion of mercury. Various such bases or vehicles have been used in our laboratory, the selection of which was based on the following objects:—(1) the mercury absorbed by the support should not be rendered inactive; (2) the vehicle used should increase the surface of evaporation of mercury; (3) the vehicle itself should not add much to the cost of the preparation, and (4) since these preparations are bound to be used on a large scale their manufacture on a commercial scale should be without any great technical difficulty.

Various preparations of mercury were made and used by the present author, amongst which were the tin-mercury, and copper-mercury amalgams. Mercury dispersed and enclosed in porous paper strips and the one dispersed in inert vehicle was also used. It was soon apparent that very few of the preparations fulfilled the above conditions. In amalgams a substantial part of the mercury was soon rendered inactive unless the concentration of mercury in it was high. Of the chief metals which amalgamate with mercury, copper is least soluble in it.[3] At the same time mercury adheres to its surface so that copper mercury surface amalgam would have served as an ideal material for the purpose of preserving grain and hence such copper plates coated with mercury were used by me in the first instance (Dole, *loc. cit.*). It was considered at the time that by the use of such surface copper amalgams, a small quantity of mercury would suffice for preservation of grain. But since then it was observed that these copper plates were rendered inactive after using them for few days. This inactivity is explained by the gradual dissolution of mercury by the copper and consequent lowering of vapour pressure of the mercury, which ultimately becomes zero if the quantity of mercury employed is less than its solubility in the copper. This is illustrated by an experiment which was carried out from 2–4–1943 to 1–5–1943.

Three copper plates measuring 10 × 5 cm. and weighing about 2·5 gm. each were coated on both sides with mercury. The amount of mercury adhering to the surface of copper plates was not uniform. Its weight varied

[3] Ramsay, 1889, **55**, 521.

from plate to plate. These plates were then placed in a well ventilated room, and they were loosely covered with an inverted glass bell-jar so as to prevent the deposition of any dust particles on the surface of the mercury but to allow the free diffusion of any vapour that might be formed by the mercury surface on the copper plate. The third copper plate with mercury coated on it was used as a control and placed in an empty desiccator of about one litre capacity, covered with a tightly fitting lid. This was done with a view to find out the loss of mercury from the plate in an enclosed space. The following table gives the losses that occurred from time to time in the weights of the different plates:—

TABLE I

	Plate No. I (Kept under a bell-jar with free diffusion)	Plate No. II (Kept under a bell-jar with free diffusion)	Plate No. III (Kept in an enclosed space)
	Grams	Grams	Grams
Weight of copper plate	2·7616	2·7416	2·7244
Weight of copper plate with mercury	3·6882	3·4272	3·5910
Weight of mercury on plate ..	0·9266	0·6856	0·8666
Weight of plate at the end of 32 days	3·5550	3·3466	3·5892
Weight of mercury left on the plate after the evaporation ceased	0·7934	0·6050	0·8648
Loss of mercury	0·1332	0·0815	0·0018
Loss of Hg / Mercury coated	$\frac{0·1332}{0·9266} = 0·1437$	$\frac{0·0815}{0·6856} = 0·1473$	$\frac{0·0018}{0·8666} = 0·0026$
% of Hg lost	14·37%	14·73%	0·26%

This shows that a substantial quantity of mercury is retained by copper under the most favourable conditions of evaporation of mercury.

It may be mentioned here that if an attempt is made to coat an increased quantity of mercury on the copper plates to compensate for the mercury held by them the copper plates lose their tensile strength and crumble to pieces.

TABLE II. *Loss of mercury of the above plates with time*

No. of days after which the loss was noted	Total loss of Hg in grams		
	Plate No. I	Plate No. II	Plate No. III
3	0·0	0·0327	0·0000
6	0·0459	0·0781	0·0003
9	0·0518	0·0811	0·0009
13	0·0656	0·0810	0·0015
17	0·0877	0·0809	0·0015
20	0·1221	0·0813	0·0016
23	0·1331	0·0815	0·0017
27	0·1331	0·0814	0·0018
32	0·1332	0·0813	0·0018

The temperature of the room in which the plates were kept was subject to varying atmospheric conditions, no attempt being made to keep it at constant temperature. No mathematical relationship can therefore be traced in the results obtained, but it can be seen that the losses in weight are obviously much greater in the earlier days than those after that period. After the first fortnight the losses in weight dwindle down materially. The plate No. III which was kept in an enclosed space does not show any appreciable loss. The slight difference in weights manifested is probably due to the loss of mercury vapour, required to saturate the one litre space of the desiccator; a very small part of it may also be due to exposure during the weighing operations.

One valuable inference that can be drawn from these observations of the negligible loss of mercury from this plate kept in an enclosed space is that a very small quantity of mercury will be sufficient for the preservation of grain provided the grain is stored in an enclosed space like a closed room or a metal storage tank. The quantity of mercury required for each place of storage can be calculated from the temperature of the room and the amount of enclosed space in which the grain is stored, irrespective of the weight of the grain stored.

A similar study of the loss by evaporation of mercury dispersed in finely precipitated calcium carbonate was also made. A powder containing 50% of mercury dispersed in it was prepared and one gram of the powder was placed in each of two watch glasses, and the powder containing the dispersed mercury was spread over an area of about 25 sq.cm. so that it formed a layer of about one millimetre in thickness. The watch glasses were then kept under cover in the manner described previously. The results of this experiment are given in the following table. The experiment was carried out from 2-4-1943 to 31-5-1943 and final weights were taken on 13-9-1943.

TABLE III

Wt. of dispersion No. I—1·0003 grams			Wt. of dispersion No. II—1·0008 grams		
No. of days	No. I	No. II	No. of days	No. I	No. II
3	0·0156	0·0168	23	0·0753	0·0690
6	0·0326	0·0331	27	0·0817	0·0742
9	0·0443	0·0438	32	0·0879	0·0773
13	0·0557	0·0527	62	0·1070	0·0938
17	0·0639	0·0597	164	0·3973	0·3916
20	0·0700	0·0649			

The results indicate that the loss of mercury is gradual and about 80% is lost from 25 sq. cm. of evaporating surface in a period of five months. The comparison of these results with the results of the copper mercury surface amalgam indicates that the evaporation of mercury from the dispersion is approximately four times as rapid as that from the surface of the amalgam. The evaporation of mercury in dispersions however would naturally depend upon the area of the evaporating surface which again is dependent on the fineness of particles of mercury. Now if the results of this experiment are studied mathematically one or two important points are brought out. The rate of evaporation of mercury from a constant surface at a constant temperature in an open atmosphere would be given by the equation $\frac{dx}{dt} = Ks\ (a-x)$ where a is the original amount of mercury, x is the amount lost and s, the surface area of evaporation, K is the evaporation constant depending upon the latent heat of evaporation, etc. On integration we get:

$$sK = \frac{1}{t} \times \log_e \frac{a}{a-x}.$$

This equation possesses the form of an equation of an unimolecular chemical reaction. On calculating the values of sK from the figures in Table III it is found the values are gradually falling. If this be taken into account it can be concluded that the evaporating surfaces are gradually diminishing. If the dispersions are thinly spread then they can be made to conform to the above equation. Similarly it may be expected that all the mercury contained in the dispersions will evaporate in due course. This expectation may very likely be fulfilled since the watch glasses with the powder, when weighed after a period of five and half months, showed a loss of 80% of the mercury.

These experiments indicate that it is possible now to assess the value of any mercury preparation for using it as a preservative, especially if the dimensions of the godown and the prevalent temperatures therein are known. By this method it is possible to measure and compare the efficiency of mercury preparations for preserving grain in storage in actual practice.

The next important point which has to be decided is the least quantity of mercury required for controlling the attack of insect pests in stored food grains. Mercury has definite vapour pressures at definite temperatures and these vapour pressures are extremely small at ordinary atmospheric temperatures. Data concerning these vapour pressures was given in my earlier paper (*loc. cit.*). Mercury vapour is mono-atomic and one can

calculate from these vapour pressures the equilibrium concentrations of mercury at different temperatures using the ordinary gas laws. Thus:—

Temperature C.	15°	16°	17°	18°	19°	20°	25°	30°	35°
Equi. con. per cu. meter in mgm. mercury	8·8	9·4	10·2	11·0	12·0	13·0	20·0	31·4	47·0

One cubic metre of space would hold about the following quantities of different grains:—

Name of the grain	Rice	Wheat	Jowar	Bajari	Turdal	Val	Vari
Weight of the grain in kilograms ..	930	815	850	850	860	818	890
Interstitial space in between the grain..	36%	38%	34·5%	34%	38·5%	37%	32%

Interstitial space in the grain has also been calculated in the above table to give a clear conception of the empty space available for the diffusion of mercury vapour around the grains. This data would give only a rough idea of the amount of grain stored in one cubic metre and the exact amount will depend upon on the mode of packing the grain. So it is expected that about 10 to 12 mgm. of mercury per cu.m. of grain plus 20 to 32 mgm. per cu.m. for the empty space above, is the theoretical quantity required if the grain is enclosed in a closed space at about 25° to 30° C. But in practice allowance has to be made for various factors. If the grain is not stored in enclosed space, large losses of the vapour are expected by diffusion. Similarly, allowance will have to be made for the improper diffusion, especially with grains of small interstitial space. Therefore, in the previous paper (*loc. cit.*) one gram of finely dispersed mercury was recommended for 240 lb. (110 Kilos.) of grain. This expectation was actually tested in the present investigation by following experiments.

In the present investigation I have used a dispersion of mercury in calcium carbonate for the purpose of determining the minimum quantity of mercury required for protecting stored food grains from an attack of insect pests. The use of a dispersion instead of the mercury coated copper, or dispersed mercury deposited between two ribbons of porous paper was resorted to, because my study of the physical factors of the formation of mercury vapour has shown that such a dispersion or dust would prove more efficacious than the other two preparations used previously. The quantity of mercury to be used was first calculated from the known weight of the dispersion and this was afterwards checked by actual chemical examination agreed fairly well. The calculated quantities of dispersions added separately to each of the several samples respectively were first weighed on a chemical balance in watch glasses by the 'difference' method,

the weights of the dispersion taken for experiment being correct up to 0·5 of a milligram. The grain chosen was *Val* (*Dolichus lablab*), because this grain is easily attacked by a bean weevil (*Bruchus phæsoli*) and the difference between the state of the healthy grain and that of the insect-attacked grain could be quickly made out and also it could be secured in sufficient quantity as it was out of the range of rationed grains at Poona.

Beans already infected with eggs and insects were used in the experiment and the amount of further damage caused by the insect was estimated by counting the number of holes in the beans. 200 Grams of the beans were weighed in several lots and the whole quantity in each lot was placed in glass bottles of one litre capacity with a loosely fitting cork so as to prevent the adult insects from coming out but allowing sufficient air for the insect life inside the bottle. The samples were examined from time to time. The following table gives the summary of the results.

TABLE IV. *Beans infected with insects and eggs found on the beans*

Quantity of mercury added 17 mgm. for 200 grams of beans. Number of holes for 25 beans selected at random from each bottle found to vary between 66 to 68.

Date	Control no treatment	Sample I 17 mgm. Hg	Sample II 17 mgm. Hg
12-4-1943	66–68 holes per 25 beans	66–68 holes per 25 beans	66–68 holes per 25 beans
12-5-1943	130–140 do.	68–72 do.	68–72 do.
12-7-1943	All food material exhausted	68–72 do.	68–72 do.
12-8-1943	Beans damaged, black with mould	68–72 do.	68–72 do.

The effect of the addition of the mercury dispersion is obvious.

In the next experiment only sound beans were selected from the whole lot. Absence of holes bored by insects was the criterion used in selecting the beans. They were also examined for the presence of eggs on the beans because for our purpose the presence of eggs on the beans was desired and a few beans having eggs visible on them were deliberately included in all the different lots selected for trial. This experiment was carried out with a view to study the influence of mercury when used from the very beginning on grain coming directly from the fields for storage in the farm buildings of the cultivator. Also it is valuable from the point of preservation of seeds on the farm by the cultivator.

TABLE V. *All beans without holes, but eggs present*

Quantity of mercury added 17 mgm. per 200 grams of beans

Dates	Treatments		
	Control no treatment	Sample I preserved with mercury 17 mgm.	Sample II preserved with Hg 17 mgm.
12–4–1943	No holes	No holes	No holes
12–5–1943	Numerous holes and some adult insects, beans hole	do.	do.
12–7–1943	Innumerable holes and beans damaged	do.	do.
12–8–1943	Beans damaged. No food left	do.	do.

There can be no doubt about the effect of mercury on the eggs of the bean weevil.

The next experiment was carried out on mixed beans about 5% of which were affected by insect attack as judged by counting the number of beans with holes per 100 beans taken out at random from each bottle.

TABLE VI. *Study of the minimum quantity of mercury sufficient to preserve 200 grams of beans in one litre of space*

200 grams of beans in each bottle, affected beans (with holes) about 5% in each bottle

Treatment	Dates of observation			
	12–4–1943	12–5–1943	12–7–1943	12–8–1943
Control beans— No treatment ..	5% beans with holes	About 90% beans with holes	All beans affected. All food material exhausted	All beans blackened with moulds
17 mgm. Hg ..	do.	5% beans with holes	6% beans with holes	5% beans with holes
6 mgm. Hg ..	4% beans with holes	do.	5% beans with holes	do.
4·5 mgm. Hg ..	6% beans with holes	4% beans with holes	do.	6% beans with holes
3·5 mgm. Hg ..	5% beans with holes	5% beans with holes	6% beans with holes	5% beans with holes
1·5 mgm. Hg ..	do.	6% beans with holes	4% beans with holes	do.

It can be concluded that the damage by the insects to the beans preserved with mercury was completely checked and did not make any progress

whatsoever after the addition of the mercury dispersion. The small differences observed are considered to be merely due to such errors as usually occur in sampling at random. Even the smallest quantity of mercury used in this experiment has proved effective. The ratio of 1·5 mgm. of mercury to 200 grams of beans amounts to 0·85 gram mercury for 240 lb. of beans or approximately 8 grams of mercury to a ton of grain (1016 Kilos) stored in a space of 1800 cu.ft. (about 50 cu.m.).

The above quantity of 1·5 mgm. per 200 grams in 1000 c.c. of space is the smallest I have so far used. In order to find out whether it could be reduced still further it is intended to use chemically precipitated mercury; but since in the course of such precipitation some mercurous chloride is likely to be formed to a smaller or greater extent in association with precipitated mercury, it is intended to see the effect of mercurous chloride (calomel) on the insect pests of the stored grain. Side by side it was intended to examine the effect of other chemicals which happened to be easily available in the chemical laboratory. Out of these, potassium metabisulphite was selected because it gradually liberates sulphur dioxide, which was considered to have some effect on the stored grain pests. Sulphur was used as it is reported to be very successfully used in the field against some crop pests and fruit pests in agricultural and horticultural practice.

This experiment was tried on beans that were already affected by weevils to a small extent as in previous experiment. The results are given in the next table.

TABLE VII. 200 *Grams beans partly attacked by insects and stored in one litre bottles*

Treatment	Dates of observation			
	12-4-1943	12-5-1943	12-7-1943	12-8-1943
Control (no treatment)	5% beans affected	75–80% loss most of the beans affected	Nearly 90% loss. Very few whole beans left	Beans blackened with fungus
1 Gram $KHSO_3$ ..	do.	24% beans affected	2/3 or 66% beans eaten up by insects	About 66% affected ?
0·26 Gram HgCl ..	4% beans affected	7% beans affected	7% beans affected	7% beans affected
5 Grams sulphur ..	6% beans affected	About 60% beans affected	Nearly 90% beans attacked	All food material exhausted

The sulphur used in our experiment has proved to be ineffective. This does not however exclude the possibility that other brands of the sulphur used in agricultural and horticultural practice may prove more effective in overcoming the insect pests of stored grain. Potassium bisulphite has shown slight insecticidal activity as compared with the control but it is nothing as compared with the effect of mercurous chloride (calomel). This material is known to be non-poisonous to human beings and possesses appreciable vapour pressure at room temperatures. Its B.P. is 383° C. as compared with the 357° C. of mercury. Investigations with this preservating material will have to be further carried out in greater detail than has been possible hitherto.

In all these experiments one fact worthy of mention is that beans preserved with mercury or mercurous chloride remained intact without deterioration, but those preserved with other materials like sulphur and metabisulphite gave out a putrid odour. This was probably due to the fact that the dead bodies of the pupæ or adult weevils killed by other materials used in the experiment got decomposed, and as these dead bodies were not removed they gave out the putrid odour on decomposition. Thence it may perhaps be a useful suggestion to bear in mind when selecting insecticides for preserving grain in godowns that it is better to choose such materials as kill the eggs and larvæ rather than those that kill the pupæ or the adults of insects.

How long would the effect of mercury last if no further insect attack takes place? Stored grain which is preserved by mercury for a certain length of time should be expected to remain without any further damage even if the mercury is removed. Even if the grain preserved with mercury by killing the eggs of the insects is removed and stored in another place it should not show any damage by the insects. In order to test the correctness of this hypothesis, whole undamaged beans were selected from the lot preserved with the smallest quantity of mercury and were exposed to air for some days during which time any vapour of mercury that might have been surrounding the grains would have been driven away from them. The beans thus freed from any mercury or mercury vapour were next stored in another fresh bottle of the same type and dimensions as before and examined at intervals. The beans were unaffected for a period of more than a month and were in the same condition as before quite sound and without any larvæ appearing in the grains or in the bottle, clearly indicating that all the eggs on the beans were actually rendered incapable of developing any insect larvæ therefrom.

Summary

Different mercury preparations—amalgams, porous strips and dispersions—have been studied with a view to measure their efficiency in preserving stored grains from insect attack. Out of the different preparations studied in this investigation mercury dispersions are the most efficient. In copper amalgam only about 14·5% of mercury is available for vapourisation.

Only very small quantity of mercury is required for preservation of grain. It is possible to preserve grain at the rate of 8 grams of mercury for one ton of grain stored in a space of 1,800 cu.ft. under suitable conditions.

Mercurous chloride, a non-poisonous compound of mercury, has been also found to be useful for preserving grain.

Since mercury is acting as a fumigant all conditions which increase the efficiency of fumigants in preserving grains would also be applicable to mercury.

THE DEVELOPMENT AND SPOROGONY OF A COCCIDIAN *MYRIOSPORA GOPALAI* N.SP., PARASITIC IN THE GUT OF THE POLYCHÆTE *CIRRATULUS FILIFORMIS* KEFERSTEIN

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Received March 24, 1945

(Communicated by Dr. N. Kesava Panikkar, F.A.SC.)

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Introduction

In a previous paper (Ganapati, 1939) I described a Coccidian *Myriospora polydoræ* from the body-cavity of a polychæte worm *Polydora ciliata* Johnst. An important phase in the development of Coccidiidæ in general, namely, asexual multiplication by schizogony was not seen in this form and, as the parasites were observed only from worms collected during a particular season of the year, the possibility of this aspect of development taking place at some other time of the year could not then be verified. I had also reasons to believe that the infection by this parasite may have been a sporadic one since the hosts examined subsequently were completely free from the parasites.

During the Summer of 1939, while examining some polychætes collected from the Madras Harbour, my attention was drawn to some large Mono-

cystis-like sporozoans in the gut of *Cirratulus filiformis*. A preliminary examination of this parasite in the living condition and in sections soon convinced me that I was dealing with a Coccidian very similar in many respects to the one from *Polydora ciliata*. The parasites confine themselves to the posterior third of the gut and they are seen attacking the sub-epithelial layer of the intestine. All stages leading to the formation of the oocyst were similar to those of *M. polydoræ*, the differences being mainly of details. For a long time my attempts to get stages of the ripe oocysts containing sporocysts and sporozoites from the worm were unsuccessful, and this, as I realised later on, was owing to the fact that the oocysts escape from the host along with the fæcal matter and complete their development outside, in the sea-water. The number of sporocysts inside each oocyst varies from 8 to 16, and the sporocysts are polyzoic. Compared with *Myriospora trophoniæ* and *Myriospora polydoræ* this number is much smaller, but for reasons discussed in another part of the paper I feel justified in including the present form in the genus *Myriospora* though it is admittedly a new species. I propose the name *M. gopalai* for this Coccidian from *Cirratulus filiformis*.

When I undertook a detailed investigation of the present form, I had in my mind the hope of solving the question of schizogony which has been missed both in *M. trophoniæ* and in *M. ploydoræ*, owing to lack of material in the former, and to the sporadic nature of the infection in the latter. Though both these difficulties were not encountered in the parasite from *Cirratulus*, the problem of schizogony remains unsolved. No trace representing this phase has been encountered in the hundreds of parasites examined from hosts collected during the several months from 1939-42. I have therefore come to the conclusion that schizogony does not take place in the worm and if such a phase in the life-history is really present, it should take place in another host. The difficulties in investigating this aspect of the problem is discussed elsewhere in this paper. The present study has in the main served to confirm many of my observations on *M. polydoræ* and incidentally to get details regarding the earlier stages leading to sporogony, which were missed in that form. At the end of the paper I have discussed the systematic position of the genus *Myriospora* from a comparison of the three species so far known.

In addition to the Coccidian, *Myriospora gopalai*, *Cirratulus filiformis* harbours three other Sporozoans and an astomatous Ciliate inside its gut. Of the Sporozoans two are Schizogregarines belonging to the genus *Selenidium* while the third is a dicystid Gregarine of the genus *Sycia*. The astomatous ciliate is a species of *Anaplophrya*, probably *A. brasili*. These parasites are described in another paper (Ganapati, 1942 unpublished).

Material and Methods

The host worms are fairly abundant in the Madras Harbour boring on shells of oysters or living amidst the debris collected on sponges and tunicates. The presence of the worms could easily be detected by their bright orange colour and by their characteristically long cirri usually found waving in the water outside their burrows. They are gregarious in habitat and have the habit of collecting together in clusters. The most successful method to induce the worms to come out of the burrows is to keep the shells after proper washing in a large basin of sea-water. The worms presumably get asphyxiated in about two hours and begin to crawl out. These are picked out and kept in clean sea-water contained in finger bowls. With daily change of water the worms remained healthy in this condition for about three weeks.

The infected worms could easily be picked out from healthy ones by examining them under a microscope when under the slight pressure of the cover glass the parasites become readily visible. For the study of the parasites all the methods described in a previous paper were employed (Ganapati, 1939). In addition Feulgen's technique was tried with success for the study of the nucleus. Fixation with Flemming's, Carnoy's or Hermann's fluid followed by suitable staining was used for special purposes. For general morphological study sections of material fixed in Bouin-Duboscq's fluid stained with iron-alum hæmatoxylin were the most satisfactory.

The oocysts were collected from the fæcal matter of the worm, using a pipette with a fine nozzle, under the binocular microscope, and kept in clean sea-water or in a 5 per cent. solution of potassium dichromate. In this condition the oocysts normally completed their development in about four days forming ripe sporocysts and sporozoites.

The Merozoites

The merozoites form the starting phase in the life-history of *Myriospora gopalai*, as it develops in the gut of *Cirratulus filiformis*. How these merozoites came into being, whether directly as a result of the growth of the sporozoites, through ingestion of the ripe sporocysts by the worm, or whether they are the ultimate products of a previous asexual multiplication in another host, are facts which shall be examined in a later part of the paper, where the question of schizogony is discussed.

The merozoites are the most conspicuous stages indicating the presence of the parasite within the worm. They are large gregariniform bodies reaching a size of nearly 150 × 25 microns. The body is long, cylindrical

and broadly rounded at either extremities (Figs. 5–7). The cytoplasm is coarsely granulated throughout excepting for a small zone at one end which in fresh preparations appears clear and hyaline. In sections stained with hæmatoxylin this end stains deep showing a large number of siderophilous granules. The nucleus is a clear rounded vesicle placed in the middle of the body and containing a large rounded karyosome. The karyosome stains dark with hæmatoxylin and the nucleoplasm is clear without any chromatin. There is a distinct nuclear membrane. Scattered in the general body protoplasm are a few deeply staining siderophil grains. The parasite is covered by a thin pellicle.

The smallest and presumably the earliest merozoites were found free in the gut or attached to the intestinal epithelium (Fig. 1). They are more or less of the same size as the sporozoites (Fig. 2) measuring about 15 microns in length. In structure they show the same features as those of the full-grown merozoite except that there is no distinct nuclear membrane surrounding the nucleus. The karyosome is large and conspicuous and there is a clear halo surrounding it. The difficulty of interpreting these free merozoites in the gut as those belonging to the Coccidian is obvious when it is known that there are three other sporozoans which occur side by side with the parasite under consideration. I have based my conclusions using as criteria two unmistakable morphological features constantly present in the merozoites. These are the structure of the nucleus and the presence of siderophil grains at the anterior end of all the merozoites. The other sporozoans have their own peculiar morphological features which preclude the possibility of confusing stages of these as belonging to the Coccidian.

The young merozoites penetrate the gut epithelium before reaching their normal habitat, namely the sub-epithelial layer. I have seen a few of these merozoites actually in the act of entering the epithelium. Inter-epithelial stages were, however, very rare and I have observed such stages only on two occasions. The rarity of this stage leads me to conclude that the penetration of the epithelium is rapid and that this is the normal means by which the parasite gains entry to the primary site of infection.

Once safely lodged the merozoites begin to grow. Each merozoite is surrounded by a sheath formed of the host's connective tissue and, in sections, a clear space is visible all round the parasite (Photomicrograph 1). This space, I believe, is produced at the time of fixation and that in the living condition the parasite is in direct contact with the host's tissue and nutriment is absorbed by the general surface of the body, which is made use of for growth.

The living merozoites do not show any movement when freed from the gut and examined in a medium of sea-water or in the body fluid of the worm. They also appear quite inert in their natural surroundings.

A comparison of the merozoite of *M. gopalai* with those of the other two species, *M. trophoniæ* (Lermantoff, 1913) and *M. polydoræ* (Ganapati, 1939) brings out the following similarities. The shape of the body, the gregariniform appearance, the presence of siderophil grains at one end, and the structure of the nucleus are identical in all the three forms. The merozoites of *M. trophoniæ* are however, much larger than the other two species. Lermantoff states that the merozoites in *M. trophoniæ* reach a length of nearly 700 microns which is four to five times the average size of the present form and of *M. polydoræ*.

Another question which we have to consider at this stage is the occurrence of a sexual differentiation of the merozoites. Lermantoff (1913) states that such a difference may be present according to the shape and the relative size of the karyosome. The males are said to be more curved and to possess a smaller karyosome than the female. I have said that in *M. polydoræ* there are no constant differences between the karyosomes of the supposed male and female and that the differences consist in the structure of the cytoplasm and the relative abundance of its inclusions. This view has been further substantiated by the study of the present form. Once the merozoites get transformed into the gametocytes it is readily possible to distinguish the male and the female.

Microgametocyte

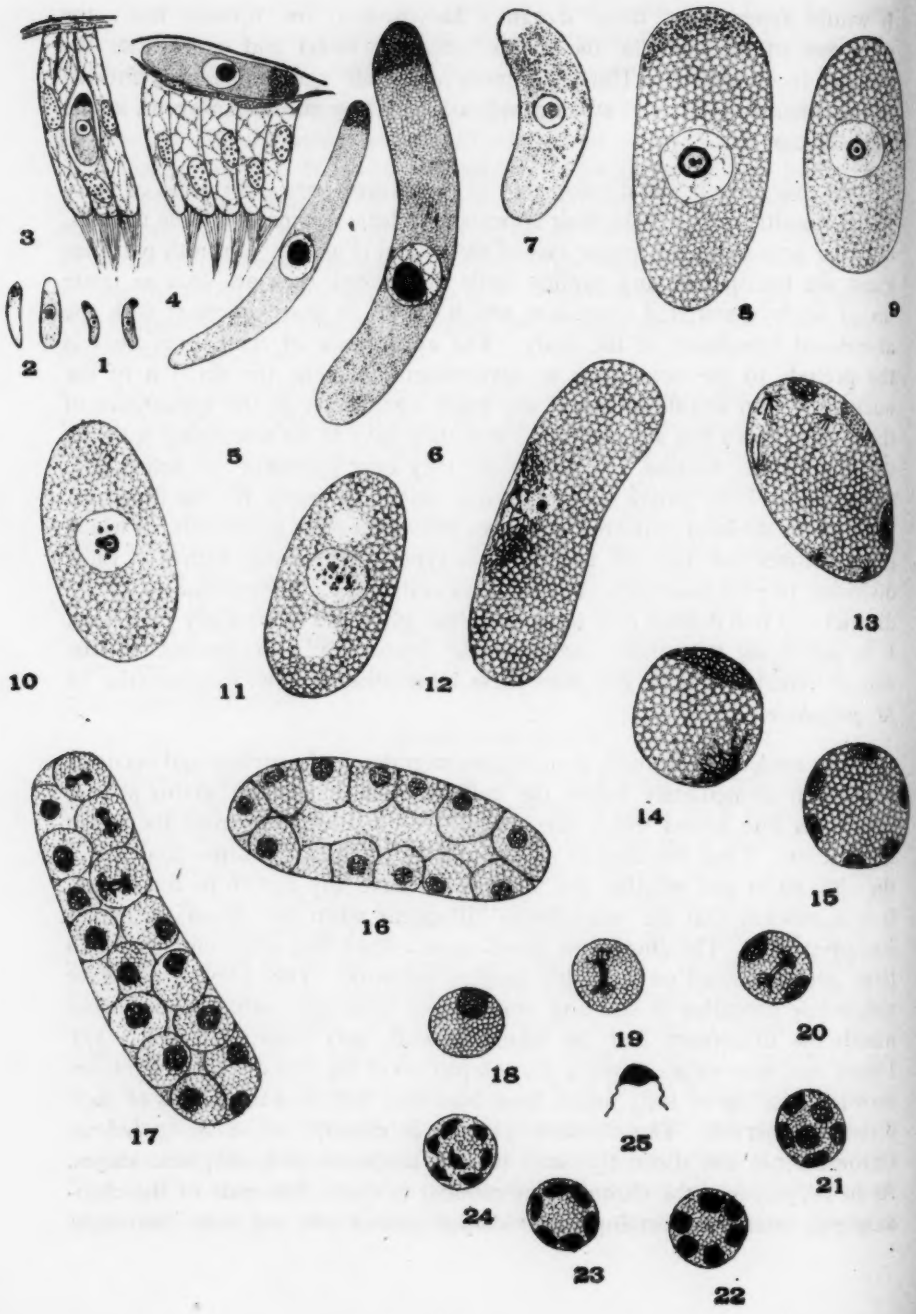
After the merozoites have attained their full growth they get transformed into the gametocytes. The transformation takes place in a manner essentially as in *M. polydoræ*. The main changes involved are a slight shortening in length accompanied by a growth in width. This is soon followed by a scattering of the siderophil grains concentrated at the anterior end into the general body protoplasm (Fig. 8). The nucleus is essentially the same as in the merozoite except for a slight increase in size. In well differentiated sections the karyosome shows an outer deeply staining rind or cortex and an inner lighter medulla. The medulla contains one or at times two small rounded bodies which reminds one of the micronucleus of *Aggregata* described by Dobell (1925). In *Aggregata* there is only a single micronucleus.

The maturation phenomena or the ripening of the male before it gets ready to form the gametes consist of some changes taking place in the nucleus. The single karyosome extrudes a number of daughter karyosomes through temporary openings formed on the cortex of the main karyosome (Fig. 10).

It would appear that these daughter karyosomes are formed from the substance of the medulla, the cortex remaining intact and entire after the process is completed. Thirty or more daughter karyosomes are formed in this manner. There is still no indication of any chromatin grains in the nuclear sap.

At this stage in the development of the parasite two clear areas of cytoplasm simultaneously make their appearance a little distance from the nucleus, at either pole along the longer axis of the animal (Fig. 11). In fresh parasites these are transparent and hyaline while in sections they are seen as made up of finely granulated cytoplasm which stands in sharp contrast with the alveolated cytoplasm of the body. The appearance of these two areas is the prelude to the next stage in development, namely, the division of the nucleus. I am unable to assign any exact significance to the appearance of these clear zones but am convinced that they have to do something with the division of the nucleus. It is possible they may represent the achromatic figures which have partly a cytoplasmic origin in many of the Protozoa. Though the division of the nucleus takes place by a form of mitosis, in which chromosomes are formed, there are no typical achromatic figures in these divisions, like the centrioles, centrospheres and asters. The chromosomes are distinct and well defined only in the anaphase stage and in the early telophase. It is significant that these characteristic areas are also present in the mature female. I have not seen these clear areas in the gametocytes of *M. polydoræ*.

The nucleus when ready for division migrates to the surface and occupies a position immediately below the pellicle. The chromatin grains at first appear as fine grains which stream into the nucleoplasm from the main karyosome. I am not able to say what part of the karyosome contributes the chromatin and whether the micronuclei have any part in its formation. It is significant that the 'micronuclei' disappear when the chromatin makes its appearance. The chromatin grains soon collect into small clumps which later get distributed on a highly tangled network. The karyosomes have meanwhile dwindled in size and only two or three are visible. The nuclear membrane disappears and the whole network gets drawn out (Fig. 12). I have not seen stages showing the completion of the first division. Parasites showing four up to sixty nuclei have been met with and the details of their division observed. The division process is exactly as in *M. polydoræ*. Chromosomes are distinctly seen in late anaphase and telophase stages. As in *M. polydoræ* the chromosome number is four. The ends of the chromosomes intercross forming spider-shaped masses and not more than eight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free ends are to be seen (Fig. 13). The nuclear divisions take place on the surface of the parasite and when these divisions are completed the nuclei occupy positions immediately below the pellicle. Each such nucleus consists of a few large globules of chromatin connected together by fine strands. The cytoplasm of the parasite forms a number of furrows, which, starting from the surface extend inwards between the nuclei. Finally each nucleus is separated with an area of cytoplasm around it (Photomicrograph 2). These secondary bodies are the real microgametocytes each of which gives rise to a few male gametes (Fig. 17). When these bodies are formed the pellicle of the male disintegrates and each one hereafter completes the development independently. Lermantoff (1913) calls these secondary bodies of the male microgametocysts; Siedlecki (1907) calls them cytomeres or microgametocyte proper, when describing similar structures in *Caryotropha* I have used the term microgametoblasts for these bodies in *M. polydoræ*. I am inclined to consider that the microgametoblasts, though in the strict sense may be compared to the microgametocytes of other Coccidians, are however, to be distinguished from the latter by adopting another nomenclature and the term microgametoblast appears to me to be the most explanatory. After the disintegration of the pellicle of the male parent the microgametoblasts get scattered to some extent and are often seen far removed and isolated from their place of origin.

Microgametogenesis

Each microgametoblast is the starting phase in the formation of 6 to 8 male gametes. The body is spherical, surrounded by a thick pellicle. The cytoplasm is closely alveolated and the nucleus lies ex-centrically. A few siderophil grains are found scattered in the cytoplasm. The single nucleus repeatedly divides into 6 to 8 nuclei (Figs. 18–24). In the later stages of division the chromatin gets more and more condensed and ultimately the whole nucleus stains homogeneously with hæmatoxylin. Each nucleus gives rise to a single gamete. The fully formed male gamete is crescent-shaped with a swollen belly and drawn out ends, each end carrying a flagellum (Fig. 25). The microgamete is composed mostly of chromatin and stains homogeneously deep with hæmatoxylin (Photomicrograph 3). When the microgametes are fully formed a spherical mass of residual protoplasm is left unused in the middle. In fresh preparations the microgametes are seen to be very active rotating inside the enveloping membrane of the microgametoblast, with the aid of the flagella. Lermantoff (1913), on the strength of Professor Dogiel's observation, states that the male gametes in *M. trophoniæ* show so much movement that the residual body inside the covering also rotates along with them. The microgamete of *M. gopalai* resembles closely

that of *Angeiocystis audouiniæ* described by Brasil (1907) from the heart-body of *Audouinia tentaculata*. The fully developed microgametes are liberated by the disintegration of the enveloping membrane of the microgametoblast and the free male gametes are often seen adhering to the surface of the female. The microgametoblasts themselves often undergo their development close to the female and this, combined with the active motility of the male gametes, facilitates the phenomenon of fertilisation.

Macrogametocyte

The female parasite gets transformed into the macrogametocyte in a manner similar to that of the male gametocyte. The merozoite destined to become the female stops growth when it reaches a length of about 120 microns, and thereafter increases in width. The cytoplasm is more coarsely granulated, the alveoli larger, and the cytoplasmic inclusions are more than in the male (Fig. 26). The nucleus has the same structure as in the merozoite except for an increase in size. The karyosome shows two regions, an outer cortex and an inner medulla. There are one or two round bodies inside the medulla corresponding to the 'micronuclei' of *Aggregata*. The karyosome extrudes a number of daughter karyosomes in a manner similar to that in the male (Fig. 27). These changes in the nuclei constitute the maturation phenomena. The nucleoplasm is free from chromatin up to a late stage in the extrusion of the karyosomes. The two clear areas of cytoplasm mentioned in the male, at either end of the nucleus, along the longer axis of the parasite are seen in the ripening female also. In the next stage the nucleus migrates to the surface. The nuclear membrane nearest the surface of the parasite gets raised up into a blunt cone-like elevation (Fig. 28). Meanwhile the chromatin makes its appearance in the form of a number of globules connected together by thin strands of the same material. The female at this stage is ripe for fertilisation or, in other words, it is now a macrogamete.

Sporogony

Very careful search of large numbers of the female parasites has not shown the actual process of fertilisation or the entrance of the male gamete into the female. I have seen the male gametes adhering to the surface of the macrogamete and the indications are that one of them succeeds in piercing through the pellicle of the female and fuses with the nucleus of the latter. The immediate result of fertilisation is the formation of a relatively thick fertilisation membrane which later becomes the oocyst wall. There appears to be a stage of rest of the zygote nucleus before division commences, and I have seen a few such stages in which the oocyst wall is present but the

nucleus still showing no signs of division. Immediately after fertilisation the cytoplasm recedes or shrinks away from the wall of the oocyst leaving a clear space between the cyst-wall and the parasite. In sections this space extends all round and the wall itself appears wavy as a result of fixation.

Though I have not seen a typical fertilisation spindle, described in many Coccidians, I was successful in getting a stage in which the zygote nucleus showed a late anaphase stage of division (Fig. 29). The chromosomes were very distinct and well defined and the karyosomes were found discarded in the cytoplasm after the distintegration of the nuclear membrane. Fig. 29 is a reconstruction of this stage from four serial sections. The chromosomes intercross at either pole of the division (Fig. 29). The number of chromosomes is four, as there are eight free ends, and this, as we have seen from the male during microgametogenesis, is the haploid number. I was unable to see an earlier stage in the division of the zygote nucleus, which is necessary to decide whether there is a reduction division at this stage as has been proved to be the case in *Aggregata* (Dobell, 1925). Since the chromosome number in microgametogenesis is haploid there is every reason to believe that the diploid number is restored at the time of fertilisation and that the first zygotic division is the reduction division. Parasites showing subsequent stages of division of the nuclei have been examined in large numbers. The details of division are the same as in the male. In all cases where it was possible to make a count the chromosome number was four. There is no indication of polymitosis. By repeated divisions 8 to 16 nuclei are formed and these come to lie on the surface immediately below the pellicle of the parasite. The nuclei are dense and rich in chromatin. The segmentation of the cytoplasm to form the sporoblasts starts from the surface as furrows which extend inwards between the nuclei. Each nucleus is ultimately separated with a bit of protoplasm around it resulting in the sporoblasts. No residual cytoplasm is left behind in the oocyst after the formation of the sporoblasts. The sporoblasts acquire a thick double-contoured wall or envelope and after its formation it was found impossible to get satisfactory fixations though all the known fixatives which have a rapid penetrating power were tried. The fixatives produced considerable shrinkage of the oocyst wall but failed to penetrate further into the sporoblasts. I had therefore to restrict my further observations on the development of the sporoblasts as revealed by an examination of fresh oocysts kept either in sea-water or in a 5 per cent. solution of potassium dichromate.

The oocyst, as stated already, is passed to the outside along with the castings of the worm, at a stage either soon after fertilisation or immediately after the sporoblasts are formed. Oocysts showing the latter condition

are more commonly met with. The gut epithelium of the host is ruptured while the oocysts are liberated and when the infection is heavy the damage produced is great. The fæcal matter of the worm was examined at regular intervals of the day and the oocysts picked out under a binocular microscope. When kept either in sea-water or in a 5 per cent. solution of potassium dichromate they completed their development in about 4 days. The walls of the oocyst and of the sporocyst are perfectly transparent and therefore it is easy to observe the changes inside by examining them at intervals under the microscope.

The wall of the oocyst is double and at one pole there is a cap-like thickening of the outer wall (Fig. 35). This pole corresponds to the micropylar end of typical Eimerian oocysts. The cytoplasm either completely fills up the space inside or in some cases a clear space is seen between the oocyst wall and the cytoplasm, at either pole, which is filled with a clear fluid. After the segmentation of the cytoplasm has taken place there is no cystal residuum and the sporoblasts completely fill up the space inside. Within the oocyst 16 sporoblasts are usually formed though, frequently, the oocyst segments even at the 8-nucleate stage producing only 8 sporoblasts (Photomicrograph 4).

Those containing intermediate numbers like 10 or 12 are extremely rare. I have examined more than 200 oocysts but the number of sporoblasts has never exceeded 16. The inconstancy in the number of sporoblasts and sporocysts led me at first to doubt whether I am dealing with more than one species of Coccidian. All evidence concerning the endogenous stages in development proved, however, that the oocyst belonged to one and the same parasite. The discrepancy in the number within the small range may be either due to the incomplete segmentation of the cytoplasm before forming the sporoblasts or it may be explained by the failure of some of the sporoblasts to complete the development.

Table I shows the measurements of 24 oocysts picked at random, 12 of which contained 8 sporocysts, and the other 12, 16 sporocysts.

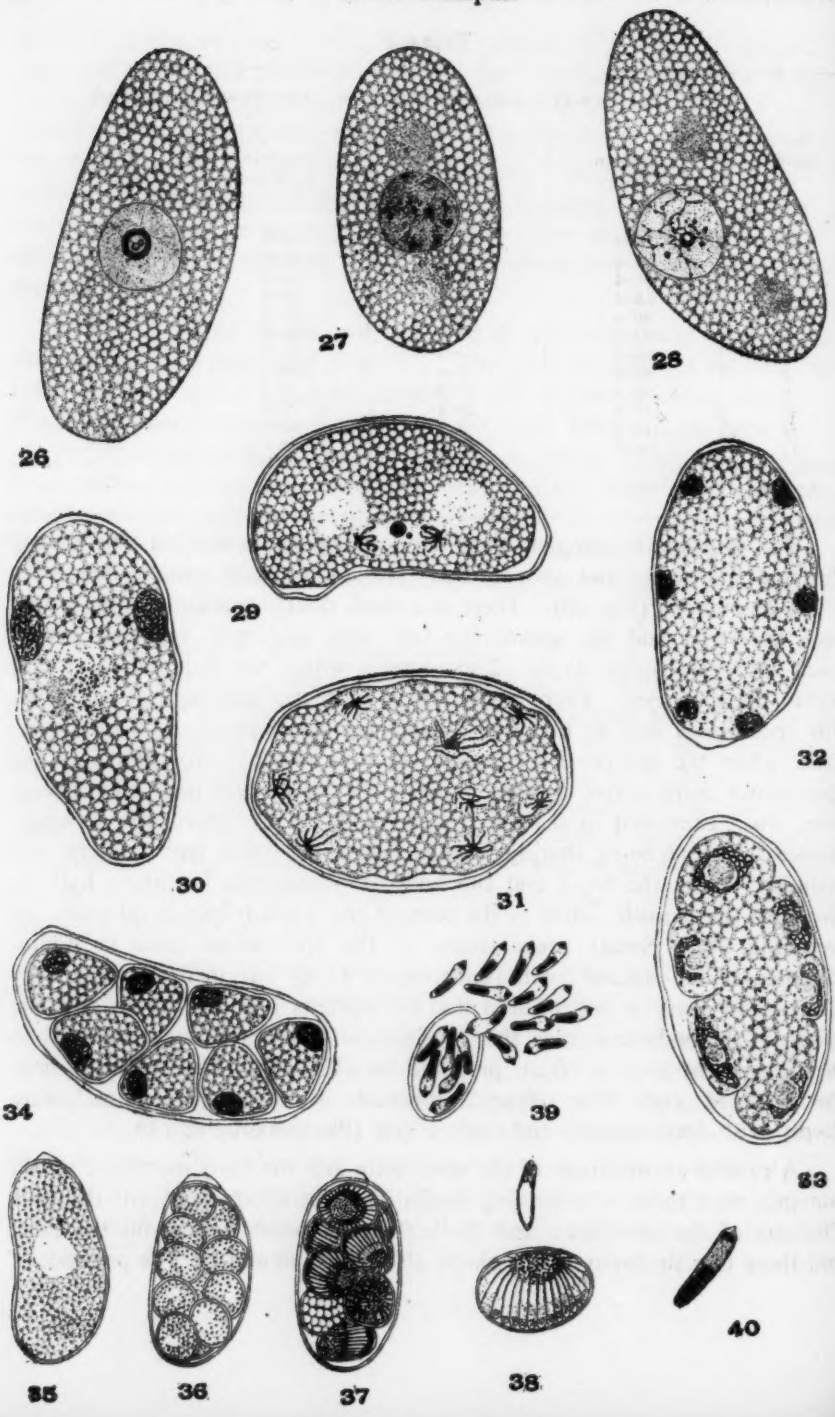
It will be seen from the table that the largest oocyst containing 16 sporocysts measures $115 \cdot 2 \times 54$ microns while the largest containing 8 sporocysts measures $90 \times 39 \cdot 6$ microns. It can also be seen that the smallest oocyst containing 16 sporocysts is only slightly smaller than the largest containing 8 sporocysts. On an average the former measure $91 \cdot 8$ microns by $53 \cdot 7$ microns, and the latter $72 \cdot 6$ microns by $42 \cdot 9$ microns. In general oocysts containing 8 sporocysts are smaller than those containing the full complement of 16.

TABLE I

Serial number	Oocysts with 16 sporocysts		Oocysts with 8 sporocysts	
	Length in microns	Width in microns	Length in microns	Width in microns
1	86·4	54·0	68·4	43·2
2	93·6	54·0	82·8	39·6
3	86·4	54·0	68·4	46·8
4	86·4	50·4	68·4	43·2
5	86·4	50·4	68·4	46·8
6	93·6	54·0	68·4	39·6
7	86·4	54·0	72·0	43·2
8	86·4	50·4	64·8	36·0
9	86·4	50·4	79·2	46·8
10	86·4	50·4	72·0	43·2
11	115·2	54·0	90·0	39·6
12	108·0	68·4	68·4	46·8
Average	91·8	53·7	72·6	42·9

The sporoblasts complete their development in about 4 days producing the ripe sporocysts and sporozoites. The sporocyst is ovoidal measuring 25 × 20 microns (Fig. 38). There is a thick double-contoured wall around each sporocyst and the sporozoites are seen arranged regularly inside. Each sporocyst forms 24 to 32 sporozoites which are disposed all round within the sporocyst. There is a spherical sporocyst residuum at one pole. The sporozoites may be liberated by applying gentle pressure on the cover-glass, when the sporocyst membrane ruptures (Photomicrograph 5). The sporozoites show active twisting movements for a short time after liberation, when examined in sea-water. The body of the sporozoite is stave-shaped, one end being sharply pointed while the other end is blunt and rounded. Near the blunt end the body is transparent for about half the length of the parasite, while at the pointed end a few refractile spherules are seen (Fig. 38). Smear preparations of the sporozoites fixed in Bouin-Duboscq's fluid stained with hæmatoxylin bring out their structure very clearly. In these it will be seen that the nucleus occupies about half the length of the body and that the position of the nucleus corresponds to the transparent zone in fresh preparations (Fig. 40). The nucleus is near the rounded end. The refractile spherules at the opposite end stain deeply with hæmatoxylin and form a cap (Photomicrograph 6).

A careful examination of the sporozoite and the early merozoite would convince that there is a striking similarity in structure between the two. The size of the sporozoite and the earliest merozoite are about the same and there is a similarity in the shape also (Figs. 40 and 1). The presence of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siderophil grains at one end is common to both, and I have no doubt these grains in the sporozoites are the forerunners of the same bodies in the merozoites. If it is imagined that the elongated nucleus of the sporozoite undergoes condensation into a rounded body it will be typically like the large spherical karyosome of the merozoite. I am inclined to consider that as soon as the sporozoites are liberated into the gut lumen this change in the structure of the nucleus takes place.

Infection of the worm presumably takes place by the ingestion of the ripe sporocysts which liberate the sporozoites into the gut lumen. The sporozoites develop into the merozoites and these into gametocytes and the life-cycle is repeated.

Schizogony

I have analysed (Ganapati, 1939) briefly the question of schizogony in *Myriospora polydoræ*, and tentatively concluded that such a phase may be absent in that form. My main reason in arriving at such a conclusion was the close similarity that existed between the sporozoite and the early merozoite. I had, however, to leave the question open on account of the limited material available, collected during a particular season of the year. In considering the question of schizogony the following possibilities have to be borne in mind, if we are to judge from known forms of Eimeriid Coccidia. In the large majority, schizogony takes place in the same host where sporogony is observed. The only known exception to this is *Aggregata* where there is an alternation of hosts, the schizogony taking place in a crab while the sporogony is in a cuttle-fish. A third possibility is that schizogony may take place only during particular seasons of the year as in *Orcheobius herpobdellæ* described by Kunz (1907) from the leech *Herpobdella atomaria*, where schizogony was observed only in material collected in the spring months of April and May. A fourth possibility is the occurrence of schizogony in some organ other than the one where sporogony takes place.

I have examined the host worms during the years 1939-42 at all the seasons of the year without finding any trace of schizogony. Serial sections of the different organs of the worm have also been examined with the same result. The only other possibility is an alternation of the host as in *Aggregata*. In the case of *Aggregata* it is well known that crabs form a major constituent in the diet of the cuttle-fish and it is quite natural to look for the alternative host in the crustacean. With this in mind I examined the gut of several worms to find out their feeding habits. The worm is essentially a vegetable feeder and the intestine contained only large quantities of minute diatoms and algæ with an occasional foraminiferan shell, which

might have got in by accident. This made the problem all the more difficult. It is beyond all reason to imagine that schizogony may take place in an animal larger than the worm itself and that the merozoites which are the end products of schizogony may have a free existence before entering the worm. The possibility of infection by inoculation by the merozoites by an alternate host may also be dismissed as far-fetched. My observations on *M. gopalai* have served to confirm my conclusion in regard to *M. polydoræ* that there is every indication to show that schizogony is absent. The peculiar method of microgamete formation by which an unusually large number of male gametes are produced by a single male and also the large number of sporozoites inside the oocyst are factors which would compensate for the absence of schizogony.

The Genus *Myriospora*

Lermantoff in the year 1913 created the genus *Myriospora* to accommodate a polysporocystid Eimeriid Coccidian which he discovered in the heart-body of a polychæte *Trophonia plumosa*. Among the characters which he gives as diagnostic of the genus are the large and gregariniform merozoites, the peculiar method of microgamete formation from secondary bodies produced as a result of the breaking up of the male parasite, and the development of a large number of sporocysts within the same oocyst, each sporocyst containing several sporozoites. An asexual method of multiplication by schizogony was not observed in the form. The extremely large size of the merozoites, the formation of a large number of sporocysts and sporozoites within the same oocyst and the absence of schizogony, so characteristic of the majority of known Coccidians, made Lermantoff doubt whether *M. trophoniæ* is a Coccidian at all or whether its proper place should not be among the Gregarines. There are, however, a few typical unmistakable Coccidian features in *Myriospora*. These are, its intra-cellular habitat during the early part of development, the widely separated nature of the gametocyte when producing the gametes, the production of numerous small male gametes composed mostly of chromatin and the development of the female parasite into a single macrogamete which in its turn gives rise to a number of sporocysts. The failure to see the schizogony is not unique in *M. trophoniæ* when it is known that of the six genera in the subfamily Aggregatinæ to which *Myriospora* belongs, schizogony has been observed only in two. In *Angeiocystis* from the Polychæte *Audouinia tentaculata* schizogony has not been observed.

Nearly twenty-five years after Lermantoff's original description of *M. trophoniæ*, I rediscovered a Coccidian in the body-cavity of *Polydora ciliata*

which showed some striking resemblance to *M. trophoniæ* in all its developmental features and described it as *M. polydoræ* (Ganapati, 1939). The sporocysts of *M. polydoræ*, while being polyzoic as in *M. trophoniæ*, are not so numerous in the oocyst, as in the type species. The general course of development and morphology, however, left no doubt as to its position in the genus *Myriospora*.

The Coccidian which I have here described essentially differs from the other two species in the fewer number of sporocysts developed within the same oocyst. This number is more definite, varying within a small range of 8 up to 16. The sporocysts are polyzoic as in the other two species. Schizogony was not observed to take place in this form also though the investigation has been more thorough and comprehensive extending over a period of three years.

Reviewing the genus as a whole we find that it occupies a unique position among the Coccidiidæ, its nearest ally being *Caryotropha mesnili*, described by Siedlecki (1907) from the spermatogonial cells of another polychæte *Polymnia nebulosa*. The resemblance between the two forms is strikingly seen in the microgamete formation. *Caryotropha*, however, does not possess large Monocystis-like merozoites, and the oocyst contains only about 20 sporocysts, each sporocyst having 12 sporozoites. Asexual reproduction by schizogony has been observed in *Caryotropha*, to take place in the same host where sporogony occurs.

Two other forms which may invite comparison with *Myriospora* as regards microgametogenesis are *Dorisiella scolelepidis* (Ray, 1930) from the polychæte *Scolelepis fuliginosa* and *Merocystis kathæ* (Patten, 1935) from the whelk *Buccinum undatum*. In both these the number of male gametes formed by a single parasite is not so large as in *Myriospora*. It is also not possible to extend the comparison beyond this similarity.

Hoare (1933) suggesting a new classification of the family Eimeriidæ bases the division of the sub-families according to the number of sporocysts formed in the oocyst, and these into genera differing from each other in the number of sporozoites in each sporocyst. The number of sporocysts in the oocyst is a constant factor in all the genera of the Eimeriidæ except the forms belonging to the group Polysporocystidea comprising of the two sub-families Barroussinæ and Aggregatinæ, where this is a variable factor. The polysporocystid *polyzoic** group includes forms ranging from monozoic sporocyst genera like *Barroussia* and *Echinospora* to polyzoic sporocyst

* The term polyzoic is used to denote the total number of sporozoites in the oocyst and not in each sporocyst.

forms like *Myriospora*. The genera *Merocystis, Pseudoklossia, Aggregata* and *Caryotropha* represent the intermediate forms in the series. Gousseff (1937) has created a new sub-family Yakimovellinæ to include a form *Yakimovella erinacei* from a hedgehog (*Erinaceus europeusp*). The oocysts of this form are octosporocystid and polyzoic. The sub-family is placed between the tetrasporocystid Eimeriinæ and the polysporocystid Barroussinæ. In creating the new sub-family Gousseff has made provision for hypothetical forms which are heccaidecasporocystid and heccaidecazoic or polyzoic. If this system is adopted it is apparent that *M. gopalai* may fit in either into the sub-family Yakimovellinæ Gousseff or it may admirably serve to take the place of Gousseff's hypothetical form which is heccaidecasporocystid and polyzoic, leaving out of consideration those rare cases where the oocyst may contain a number between 8 and 16. To say the least adopting this criterion alone, namely, the number of sporocysts in the oocyst, in determining the systematic position of any particular form of Coccidian sounds most unnatural taking into consideration other morphological and developmental similarities in the endogenous phase of the life-history. I therefore feel that while in those forms where the sporocyst number in the oocyst is a constant factor, Hoare's system of classification may be the most convenient, it is not so in the case of the polysporocystid sub-families where the sporocyst number is variable. In all such cases it is necessary to know the endogenous life-cycle before a particular form is assigned to the generic rank. A study of the three species of *Myriospora* (two by me and one by Lermantoff) has clearly shown that while the endogenous development is identical in all the three, there is considerable variation in the structure of the oocyst especially in the number of sporocysts formed, though, in all of them the sporocysts are polyzoic.

Myriospora gopalai Spec. Nov.

Diagnosis.—Merozoites large, elongated, with siderophil bodies at one end. Microgametocysts long and cylindrical breaking up into 60-80 microgametoblasts, each of which forms 6-8 male gametes. Microgametes crescent-shaped and biflagellate. Macrogametocyte shorter and broader than the male. Oocysts ovoidal, measuring 90-100 by 50-60 microns. The wall of the oocyst has a cap-like thickening at one pole. 8-16 Sporocysts are formed in each oocyst. Sporocysts ovoidal measuring 25 × 20 microns. Sporocysts polyzoic producing 24-32 sickle-shaped sporozoites. A sporocyst residuum present.

Schizogony not known.

Habitat.—Gut of *Cirratulus filiformis*, Madras Harbour, Madras.

Summary

1. The development and sporogony of a new Coccidian, *Myriospora gopalai* n.sp., are described, from the gut of a polychæte *Cirratulus filiformis*.

2. Merozoites are large and elongated with characteristic siderophil bodies at one end.

3. Microgametocyte is long and cylindrical producing 60–80 microgametoblasts. Each microgametoblast develops independently, forming 6–8 crescent-shaped biflagellate male gametes.

4. The females are shorter and broader than the males.

5. The chromosome number during microgametogenesis and in the nuclear division of the oocyst is haploid and it is believed that the diploid number is restored at the time of fertilisation. The first zygotic division is probably a reduction division.

6. Oocysts are ovoidal with a characteristic thickening of the wall at one end. 8–16 Sporocysts are formed by each oocyst. Sporocysts are polyzoic containing 24–32 sporozoites.

7. Schizogony was not observed and this question is discussed.

8. The systematic position of the genus *Myriospora* Lermantoff is discussed and it is pointed out that the criterion of the oocyst structure alone cannot be emphasised in separating the polysporocystid Eimeriid genera.

Acknowledgments

I wish to express my deep sense of gratitude to Professor R. Gopala Aiyar for his constant encouragement and constructive suggestions. I am also indebted to the Syndicate of the University of Madras for giving me all facilities for carrying out this work.

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Explanation of Text-Figures

All figures were drawn from sections with the aid of a camera lucida at stage level, with Zeiss apochromatic oil immersion objective and compensating oculars. Unless otherwise stated, the material was fixed in alcoholic Bouin-Duboscq and the sections stained in Heidenhain's iron-hæmatoxylin. The magnifications given are those at which the drawings were made but figures (1-40) have been reduced to a little less than one half.

Fig. 1. Section of two merozoites free in the gut lumen. ×1800.

Fig. 2. A sporozoite and an early merozoite (fresh preparation). ×1800.

Fig. 3. Section of gut to show an intra-epithelial merozoite. ×1800.

Fig. 4. A merozoite in the sub-epithelial tissue. ×1200.

Figs. 5 & 6. Two stages in the growth of the merozoite. ×1200.

Fig. 7. A fresh merozoite. Note the clear zone of protoplasm at the anterior end. ×800.

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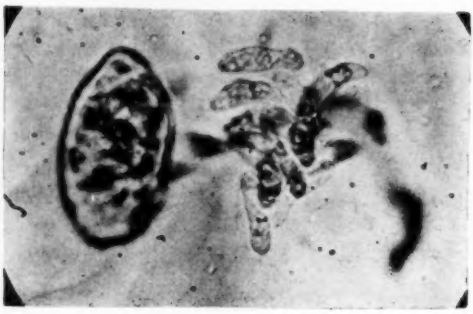

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Fig. 8. A merozoite transforming into the gametocyte. Note the siderophilous grains at the anterior end spreading into the cytoplasm and also the structure of the nucleus. ×1800.

Fig. 9. A male gametocyte. ×1800.

Figs. 10 & 11. Maturation of the male. Note changes in the nucleus and the clear areas of cytoplasm at either pole of the nucleus. ×1800.

Fig. 12. A male showing the nucleus dividing on the surface. ×1800.

Fig. 13. A later stage of the male showing a few nuclei in different stages of division. ×1800.

Fig. 14. A cross-section of a male at the above stage. ×2400.

Fig. 15. A cross-section of a male at a later stage to show the nuclei arranged on the surface. ×1800.

Fig. 16. Formation of microgametoblasts. ×1800.

Fig. 17. Male showing the fully formed microgametoblasts. ×1800.

Figs. 18-24. Different stages in microgametogenesis. ×2400.

Fig. 25. A male gamete. Note the two flagella. ×2400.

Fig. 26. A macrogametocyte. ×1800.

Fig. 27. A ripening female. Note changes in the nucleus. ×1800.

Fig. 28. A macrogamete. ×1800.

Fig. 29. Oocyst showing division of the zygote nucleus. ×1800.

Fig. 30. A later stage of oocyst showing two nuclei. ×1800.

Fig. 31. A multinucleate oocyst showing the nuclei in various stages of division. ×2400.

Fig. 32. Oocyst showing the resting nuclei on the surface. ×1800.

Fig. 33. Oocyst showing formation of sporoblasts. ×1800.

Fig. 34. Oocyst with the sporoblasts inside. ×1800.

Fig. 35. A fresh oocyst. Note the cap-like thickening of the wall at one pole. ×600.

Fig. 36. Oocyst with sporoblasts inside (fresh). ×600.

Fig. 37. A ripe oocyst with sporocysts and sporozoites (fresh). ×600.

Fig. 38. A ripe sporocyst. Note the sporocyst residuum (fresh). ×1200.

Fig. 39. Sporocyst showing escape of sporozoite after rupture (smear). ×1200.

Fig. 40. A sporozoite. ×2400.

Explanation of Plate XI

Photomicrograph 1. A merozoite. Note the outpushing of the epithelium by the growth of the parasite. ×about 600.

„ 2. A male showing the microgametoblasts. ×about 1200.

„ 3. A few microgametoblasts showing the microgametes. × about 1200.

„ 4. An oocyst which is ruptured showing 16 sporocysts (fresh). about × 1200.

„ 5. A ruptured sporocyst showing the escape of the sporozoites. ×about 1200.

„ 6. A sporozoite (smear).

LOIMOS SECUNDUS (MONOGENEA, TREMATODA) FROM THE GILLS OF THE COMMON INDIAN DOG-FISH (*SCOLIODON SORRAKOWAH*)

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Received May 15, 1945

ONLY two specimens of this interesting monogenetic trematode were obtained by one of us (G. D. B.) from the gills of a preserved common Indian dog-fish (*Scoliodon sorrakowah*) at Rangoon, 1925. Study of the worms revealed that they represent a new species of the genus, *Loimos*.

Loimos secundus (Chauhan and Bhalerao, 1945)

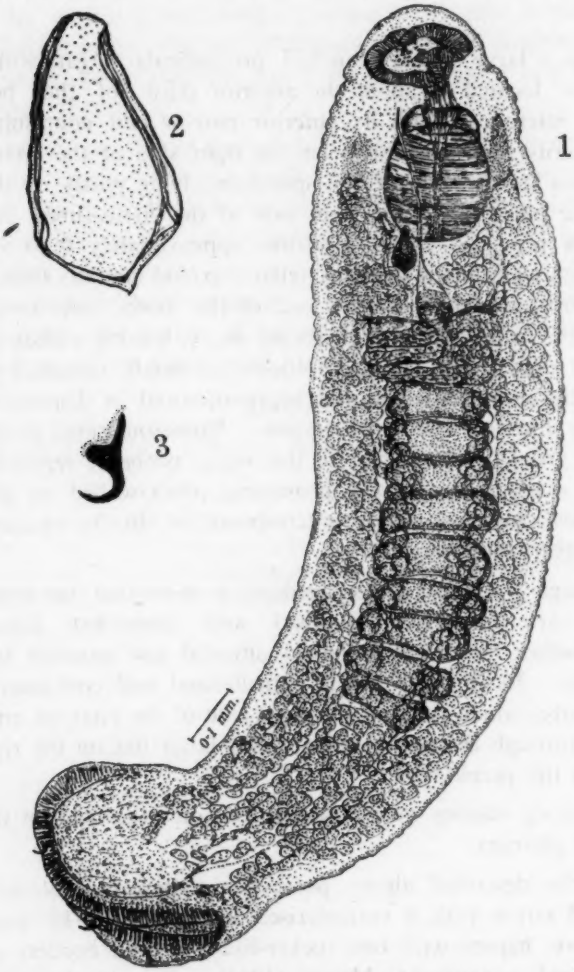
(Syn. *Tricotyle secundus* Chauhan and Bhalerao, 1945)

(Text–Figs. 1, 2 and 3)

Body flat, elongate, tapering anteriorly, broadest in the middle, with a spatula-shaped posterior haptor which is distinctly set off from the body. The parasite measures 1·61–1·63 mm. in length and 0·38–0·48 mm. in greatest width. Anteriorly, the conical head-lobe is separated from the body by slight, narrow, lateral constrictions. Mouth subterminal, guarded by two somewhat inconspicuous sucker-like structures having well-defined radial muscles and semicircular apertures. It leads posteriorly into a distinctly marked pre-pharynx provided with circular muscles and controlled by lateral muscular bands.

Pharynx large and elongate, oval, highly muscular with characteristic transverse muscular bands, measuring 0·18–0·20 × 0·14–0·16 mm. Oesophagus not seen. The two simple and unbreached intestinal cæca pass slightly upwards and then turn posteriorly, proceeding behind along the testes and terminating at a short distance in front of the posterior end of the body.

At the posterior end of the body is a peculiar disc-like haptor, without septa, measuring 0·33–0·35 mm. in diameter. It is a bipartite structure, with dorsal and ventral sucker-like, semi-circular, convex, marginal borders, directed posteriorly. These membranous borders are ribbed on their inner surface with thickenings which extend in an antero-posterior direction and probably represent the terminal portions of numerous longitudinal muscle



FIGS. 1-3.—Fig. 1. Ventral view of *Loimos secundus*. Fig. 2. Egg of *Loimos secundus*. ×450. Fig. 3. Haptoral hook of *Loimos secundus*. ×450.

fibres, which extend anteriorly into the body. On the posterior dorsal margin of the haptor are seen a pair of transverse circular folds. They probably represent the two dorsal shallow, cup-shaped structures observed by Manter (1938) in *L. scoliodoni*. The haptor possesses a pair of large widely-separated hooks which have a heavy small truncated root, a long and slender root and a sharply curved blade. The total average length of

a hook is 0·07 mm. Small marginal hooklets were not observed on the haptor.

Ovary is a large, much branched, pre-testicular organ with numerous sinuous tubes, located at about the anterior third of the body. It is median and intercæcal, with its anterior narrow end continuing into the oviduct. Uterus runs anteriorly, on the right side of the cirrus tube. A single egg was seen in one of the specimens, lying partly on the posterior border of the pharynx, to the right side of the cirrus-tube. It is conical, with a small posterior spine, measures approximately 0·14 × 0·07 mm. and has a highly chitinised shell. Vitellaria extend laterally from the middle of the pharynx near the posterior end of the body, becoming confluent posterior to the testes and anterior to the ovary, leaving a clear tract along the intestinal cæca. The vitelline follicles are small, spherical or elongate and are partly supra-testicular and supra-intestinal in disposition. Transverse vitelline ducts are clearly observable. Numerous small glands, situated in two close groups just anterior to the ovary, probably represent the shell glands. The vagina is not very prominent, thick-walled or muscular as in *L. scoliodoni*, but is a simple antero-posterior slit-like opening, situated postero-lateral to the pharynx.

Testes large, 9–10 in number, median, post-ovarian, tandem and intercæcal. They are transversely elliptical and somewhat lobed bodies. Cirrus-sac median, small, flask-shaped, situated just anterior to the shell gland complex. Its anterior end is cuticularised and continues as a long and slender tube, which runs on the right side of the pharynx and opens to the exterior, through the genital pore. This latter lies on the right side, in the region of the prepharynx.

The excretory vesicles were observed, one on either side of the anterior region of the pharynx.

The species described above, possessing a pair of anterior suckers, well-developed cirrus with a cuticularised long tube, 9–10 testes, much branched ovary, haptor with two sucker-like marginal borders and a pair of hooks, would, according to Manter (1944), belong to the genus *Loimos* MacCallum, 1917 (Syn. *Tricotyle* Manter, 1938). The genus *Loimos* contains three species, *L. salpinggoides*, *L. scoliodoni* and *L. secundus*. The last species resembles *L. salpinggoides* in the possession of nine testes and probably in the shape of egg and cirrus tube, but differs from it in having a single pair of anterior suckers, absence of marginal hooklets on the haptor, the position of genital pore and the egg and in the relative position of the terminal portions of genitalia. It resembles *L. scoliodoni* in the number of

anterior suckers, but differs from it in the structure of vagina, more anterior position of the genital pore, a longer cirrus-tube, absence of marginal hooklets on the haptor and in the lack of three well-marked anterior lobes. The number of testes is also different, being 9–10, whereas in *L. scoliodoni* these are only five.

We accept the creation of the sub-family *Loimoinæ* for the genus *Loimos*, but suggest its transfer from the family *Monocotylidæ* to the family *Microbothriidæ*, on the grounds that members of the sub-family *Loimoinæ* differ from Monocotylids, in the total absence of septa from the haptorial disc, number of testes and in the nature of ovary and anterior haptors. They more nearly resemble Microbothrids, in the nature of anterior haptor consisting of suckers, the nature of buccal cavity and œsophagus, and in the posterior haptor being non-septate.

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THE INFLUENCE OF CHEMICAL MANURES UPON 'WHITE ROT' OF *ALLIUM*

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Received April 18, 1945

White rot of *Allium* caused by *Sclerotium cepivorum* Berk. is a serious disease of *Allium cepa* Linn. (onion), *A. fistulosum* Linn. (Welsh onion), *A. porrum* Linn. (leek), *A. sativum* Linn. (garlic), and *A. ascolonicum* Linn. (shallots), but *A. cepa* Linn. suffers the most. The primary symptom of the disease is wilting with yellowing of the foliage. First of all the oldest leaves turn yellow and fall over, followed by others which also collapse. Such plants will come off the ground by even a gentle pull and on examination it would be found that almost all the roots are destroyed. The fungus persists during winter in soil by sclerotia which germinate late in the spring and thus bring about the infection of the new crop. The sclerotia can remain viable in soil for more than five years. In European countries and America it becomes very destructive malady as the fungus thrives best at moderately cool temperatures with moderate soil moisture. Sowing onions year after year in the infected soil aggravates the disease. The principal means of wide dissemination of the fungus is the transportation of infected bulbs or plants, or by containers of the same.

The disease is of widespread occurrence. Berkeley[1] first observed the fungus and named it *Sclerotium cepivorum* Berk. Voglino[11] recorded severe attack of leeks in Italy and renamed the fungus *Sphacelia allii*. Cotton and Owen[5] reported that the disease caused considerable damage to onion crops, shallots were markedly resistant and leeks did not appear to suffer. Caballero[4] reported considerable damage to garlic in Spain. Walker[12] found that the disease occurred on onion, Welsh onion, leek, garlic and shallot in Europe and America. Nattrass[7] reported the occurrence of the disease from Egypt. Du Plessis[6] gave an account of the disease from South Africa and reports that disinfection of soil by formalin, heat or mercuric chloride are impracticable on a large scale though in laboratory test the sclerotia succumbed to these treatments. Onion varieties showing marked resistance to *S. cepivorum* have been developed at Manchester. Nattrass[8] reported the occurrence of the disease in Cyprus and suggests that sets should only be planted from disease-free areas and cultivation of the different

species of *Allium* should be discontinued for 8 to 10 years. Bremer[3] reports the presence of the disease in Germany and also recommends a well-regulated rotation in which onions are excluded from infested fields for at least 8 to 10 years. Ogilvie and Hickman[9] found the disease widely distributed in Bristol province mainly on White Lisbon Spring onion. A soil application of a proprietary organic mercury compound in dust form containing hydroxymercuri chlorophenol with 20 per cent. organically combined mercury, before sowing, gave 56·8 and 17·9 per cent. infection at two localities respectively against averages of 86·7 and 90·4 per cent. for the corresponding untreated control plots. Ogilvie, Croxall and Hickman[10] found that early autumn sowings of onions were more severely affected by white rot than were late sowings. Brandão[2] has given popular notes on the symptoms and control of the disease affecting garlic in Brazil.

A series of experiments were carried out at Slough during the summers of 1931 and 1932 to find the effects of certain chemical manures on the incidence of the disease. All through the laboratory and field experiments 'White Spring Lisbon' onion seeds were used. Other varieties when used have been mentioned at the appropriate places.

A plot 44 × 6 ft. was divided into six equal plots, A–F, which were manured each year with superphosphate, potassium sulphate and ammonium sulphate in ratio of 6:1:2 (A), 6:0:2 (B), 6:4:0 (C), 6:4:2 (D), 6:0:8 (F), and 0:0:0 (control) respectively. Six hundred seeds were sown in each plot with (*a*) uninoculated (control) and (*b*) inoculated with *Sclerotium cepivorum*.

The same piece of ground has been used during the previous year for certain tests with *S. cepivorum* and as it turned out, had become contaminated to some extent with that fungus. The result was that both the control rows and the rows inoculated with *S. cepivorum* showed seedlings being killed off. A study of these by the ordinary cultural methods proved that the active fungus was *S. cepivorum*. The experiment was thus to some extent vitiated, but certain indications were forthcoming. These are given in Tables I and II.

Table I gives the results obtained during the first year.

The controls remained comparatively free of infection except plot F.

Results from the above table show that there was very heavy attack by *S. cepivorum* in plots A, B, E and F. It is suggestive that the two plots in which little attack took place (C and D) were those with high potash manuring.

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TABLE I

Superphosphate, K_2SO_4 and $(NH_4)_2SO_4$ ratio in the plots	Treatment	Percentage of germination	Percentage of healthy plants	
			After 40 days	After 63 days
A	*S. cepivorum*	46·7	17·0	1·2
6 : 1 : 2	Control	73·5	73·5	71·5
B	*S. cepivorum*	54·7	37·6	13·1
6 : 0 : 2	Control	71·8	71·8	70·8
C	*S. cepivorum*	62·4	59·3	59·3
6 : 4 : 0	Control	68·8	68·8	68·8
D	*S. cepivorum*	66·9	65·2	65·2
6 : 4 : 2	Control	67·8	67·8	67·8
E	*S. cepivorum*	53·7	29·4	5·4
6 : 0 : 8	Control	71·3	71·3	69·3
F	*S. cepivorum*	22·0	2·4	0·0
0 : 0 : 0	Control	69·0	59·0	54·0

The results obtained in the same series of plots during the next year are given in Table II.

TABLE II

Superphosphate, K_2SO_4 and $(NH_4)_2SO_4$ ratio in the plots	Treatment	Percentage of healthy plants	
		After 40 days	After 90 days
A	*S. cepivorum*	17·3	8·1
6 : 1 : 2	Control	24·3	20·5
B	*S. cepivorum*	16·8	14·6
6 : 0 : 2	Control	25·0	16·5
C	*S. cepivorum*	24·3	13·5
6 : 4 : 0	Control	22·6	18·6
D	*S. cepivorum*	16·0	8·6
6 : 4 : 2	Control	18·0	13·8
E	*S. cepivorum*	11·3	6·0
6 : 0 : 8	Control	15·8	7·1
F	*S. cepivorum*	16·5	4·6
0 : 0 : 0	Control	25·0	15·1

These results show that the control plots were now seriously infected, and for that reason no conclusion could be drawn.

An experiment of a similar type was carried out in which onion seed was sown uninoculated or with *S. cepivorum* on plots which were manured in the following manner:—

A. Superphosphate at the rate of 6 cwt. per acre.

B. Potassium sulphate at the rate of 2 cwt. per acre.

C. Ammonium sulphate at the rate of 2 cwt. per acre.

D. No manure.

A double series of plots of this kind was prepared, the one in dry light land and the other in rather peaty wet land. The results obtained in the two experiments are shown in Tables III and IV. In each plot 400 seeds were inoculated by *S. cepivorum* and the same number were sown as control.

TABLE III

Dry light soil

Plots and manuring	Percentage of healthy plants	
	S. cepivorum	Control (uninoculated)
A. Superphosphate	28·8	44·5
B. Potassium sulphate	34·5	45·0
C. Ammonium sulphate	29·0	40·0
D. No manure	25·0	43·0

TABLE IV

Peaty wet soil

Plots and manuring	Percentage of healthy plants	
	S. cepivorum	Control (uninoculated)
A. Superphosphate	17·6	61·0
B. Potassium sulphate	20·0	66·4
C. Ammonium sulphate	20·0	67·2
D. No manure	42·0	70·0

Both tables indicate considerable attack by *S. cepivorum*, but it is doubtful if any specific value can be attached to any of the manurial treatments as regards checking the disease.

The diminished attack on plots with high potash manuring indicated in Table I appeared to run parallel with greater resistance of the bulb tissues

to invasion. Ten healthy bulbs were selected from each of the plots, and comparable inoculations by *S. cepivorum* on the scales of these were carried out in the laboratory. The average amounts of attack produced after 12 days were as in Table V.

TABLE V

	Average area of rot caused by *S. cepivorum* on onions from different plots					
Plot	A	B	C	D	E	F
Average rot area (in cm.)	1·5	1·2	0·5	0·8	1·6	2·0

It is noteworthy that the bulbs derived from plots C and D (compare Table I) showed considerably less attack than the others.

An experiment on the effect of liming on the incidence of the disease was also carried out. Three treatments were tested, (*a*) no lime, (*b*) lime at the rate of 2 tons per acre, and (*c*) lime at the rate of 5 tons per acre. Each plot was duplicated. The results observed after 50 and 110 days are shown in Table VI.

TABLE VI

Plants (Numbers)	Days	Limbux 5 tons per acre		Limbux 2 tons per acre		No lime	
		Inoculated by *S. cepivorum*	Uninoculated	Inoculated by *S. cepivorum*	Uninoculated	Inoculated by *S. cepivorum*	Uninoculated
Autumn sown onion seedlings (30)	50	28	30	26	30	30	30
	110	26	30	25	30	20	30
"White Lisbon" onion seeds (300)	50	170	189	163	174	79	172
	110	142	189	130	172	26	172
"Blood Red" onion seeds (300)	50	115	103	104	133	122	138
	110	93	99	98	131	86	135
"Musselburgh" leek seeds (300)	50	175	189	202	206	176	185
	110	155	194	167	205	158	184
Shallots (30)	50	29	30	28	29	28	30
	110	28	29	28	29	27	30

Here it will be seen that to a marked extent a control of the disease 'white rot' of Autumn-sown "Lisbon" onion seedlings or "White Lisbon" seeds is obtained by liming at the rate of 2 to 5 tons per acre. Other species of *Allium* are comparatively not so susceptible to the attack of *S. cepivorum*.

As a matter of interest it may be added that the average weight of onion bulbs was considerably affected by the different manurial treatments in the case when the experimental plot was on light land, but where the experiment was carried out on dark peaty soil, no significant difference was seen. Sample figures are given in Tables VII and VIII.

TABLE VII

Sandy light land

	Manuring ratio of Superphosphate : Potassium sulphate : Ammonium sulphate					
Ratio	6 : 1 : 2	6 : 0 : 2	6 : 4 : 0	6 : 4 : 2	6 : 0 : 8	0 : 0 : 0
Average rate of onion bulbs after 25 weeks (in gm.) ..	42	50	22	50	73	20

TABLE VIII

	Average weight of onion bulbs after 15 weeks	
	Sandy light soil gm.	Dark peaty soil gm.
Superphosphate	4	7·0
Potassium sulphate	5	8·2
Ammonium sulphate	21	7·5
No manure	9	6·0

It will be observed from the above two tables that on the sandy light land the average size of the bulbs showed a marked response to nitrogenous manuring.

My grateful thanks are due to Professor W. Brown for his valuable suggestions and directions.

SUMMARY

1. High potash manuring showed some decrease in the attack of *Sclerotium cepivorum* on onions. This diminished attack appears to run parallel with greater resistance of the bulb tissues to invasion.

2. There was an indication from plot experiments that liming reduced considerably more the amount of 'white rot' of onion seedlings than the potash manuring.

3. The average weight of onion bulbs was considerably affected by different manurial treatments. On sandy light land, nirtogenous manuring showed a marked increase in the weight and size of the bulbs.

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DISEASES OF *PAN* (*PIPER BETLE*) IN SYLHET, ASSAM

*V. *Sclerotial* Wilt

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(With one Plate and two Text-figures)

Received April 11, 1945

(Communicated by Prof. H. Chaudhuri, D.Sc., F.A.Sc.)

I. Introduction

The *sclerotial* wilt of *pan* (*Piper betle*, Linn.) due to *Sclerotium rolfsi* Sacc. is a serious disease and causes heavy damage to the crop wherever it occurs. The disease has been found to occur principally in the villages Kumrakapan, Purangaon, Phulbari, Faua and Erangabaj. It is present during the rains and is practically absent during the other months of the year.

The damage done by the disease has been found to vary from *boroj* to *boroj*. Observations made on the *pan* crop of villages Purangaon and Phulbari showed that the percentage of deaths of the plants is between 4 to 31 per cent. in different *borojes* while in the villages Faua and Erangabaj, on an average, 12 per cent. of the plants are killed annually. In the village Kumrakapan it has been found that 5 to 21 per cent. of the plants are destroyed yearly on account of this malady.

II. Symptoms of the Disease

The *pan* plant is susceptible at any stage of growth. The plants are usually attacked at the ground level; dense white cotton-like mass of threads, the mycelium of the fungus, are seen on the stems. The fungus causes a rotting of the stem tissue at the point of attack and brings about a wilting and the ultimate death of the plants. Soon after, sclerotia, mustard seed-like bodies, are formed on the stem and also on the soil near the base of the plant. They are produced in great number. In the early stages of infection the leaves droop and the plant wilts and is ultimately killed. The bulk of the fungus mycelium remains outside the stem but hyphæ are traced

* Parts I, II and III appeared in Vol. XIX. No. 5 and Part IV in Vol. XX. No. 6 of the *Proc. of the Indian Acad. Sci.*, Sec. B.

in and among the cells of all the tissues, where they break down the middle lamella so that the cells fall apart causing a soft rot. Hyphæ are formed within the tissues both between and in the cells. Plate I shows the symptoms of the disease.

III. Incidence of the Disease

To study the incidence and progress of the disease from week to week certain *borojes* at Kumrakapan in which the disease is known to occur year

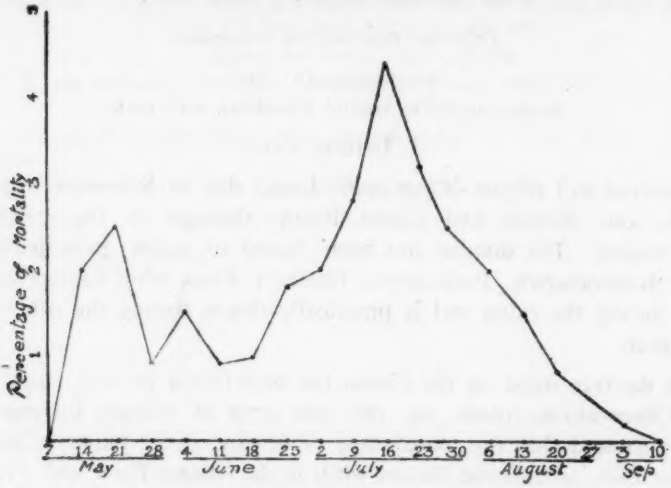


Fig. 1. Mortality of plants due to sclerotial wilt in 1940

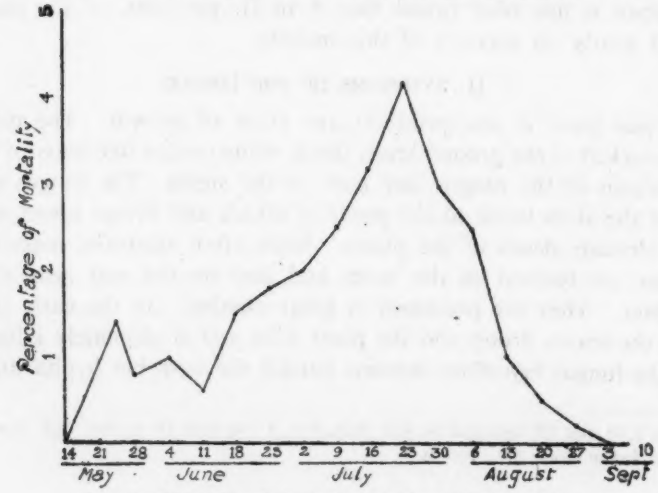


Fig. 2. Mortality of plants due to sclerotial wilt in 1941

after year were kept under observation for two seasons. The number of deaths was carefully recorded every week. Figs. 1 and 2 show the progress of the disease in a certain *boroj* at Kumrakapan during 1940 and 1941 respectively.

From Figs. 1 and 2 it will appear that the disease appears during the middle or later part of May and continues to be vigorous during the later part of June and whole of July. From the first or second week of August the rate of death of plants gradually falls off and the disease ceases by the first week of September.

Similar observations were made at Phulbari and Purangaon during the seasons 1941 and 1942. The results obtained were found almost identical with those obtained at Kumrakapan.

IV. Inoculation Experiments

The fungus was obtained in pure culture in the usual way and the following inoculation experiments were carried out with pure cultures of the fungus.

(i) *Direct Inoculation.*—Twenty-seven *pan* plants were selected in a *boroj* and a sclerotium was placed at ground level near each one of the plants. Next day hyphæ from the sclerotium were growing on them. On the succeeding day the mycelium was fairly copious and the stem under it was becoming brown. On the fifth day the surface of the stem for a length of from three to five centimetres from the place of inoculation was brown, soft and rotten and easily broken away at the nodes near the infected place. The white mycelium was running along the stem in straight lines and radiating in a white fan-shaped weft on the surface of the soil. Sclerotia were forming as small white pin-heads on the mycelium, both on the stem and soil. The upper parts of the plants showed signs of wilting on the third day and on the fifth day they had collapsed. The same fungus was re-isolated from the dead plants and it was found to be the same as the one used to inoculate the plants.

Controls were kept and all of them remained healthy.

(ii) *Soil Infection.*—A pan *boroj* was raised on a land where *pan* had never been grown before. It was divided into two equal parts; one part was artificially inoculated in September 1941, with the fungus (mycelium and sclerotia) grown on oat agar, the other part served as control. In October 1941 equal number of healthy setts of *pan* were planted on these plots and kept under constant and careful observations for the years 1941 and 1942. During this period plants of different ages wilted from time to

time producing typical symptoms of the disease. The results of the observations are recorded in Table I.

TABLE I

Summary of the infection experiments carried out by infecting the soil with S. rolfsi

Treatments	Total No. of plants	Number of deaths in 1942						
		May	June	July	Aug.	Sept.	Oct.	Total No. of deaths
Soil infected with fungus ..	240	7	24	39	15	5	..	90
Soil not infected with fungus	240	..	..	..	..	..	..	..

All the dead plants were examined and it was found that in all cases the death was due to *S. rolfsi.*

(iii) *Depth of Burial of the Fungus in the soil in Relation to Infection.*—Inoculation studies were carried out by placing sclerotia and mycelia on the surface and at 1, 2, 3, 4, 5 and 6 inches deep in the soil. Rapid infection and death of the plants were observed after 3 to 5 days when the sclerotia and mycelia were on the surface or buried one inch deep. Delayed infection was noted when the fungus was buried two inches deep. No infection resulted where the sclerotia and mycelia were buried 3, 4, 5 and 6 inches deep even after 30 days when the observations terminated. The results therefore show that the fungus is very active and readily caused infection when it was near the surface but may become inactive and incapable of infecting susceptible plants when buried 2 to 6 inches deep in the soil unless brought towards the surface by cultivation or other means.

(iv) *Conditions necessary for successful infection.*—Careful observations during the carrying out of the infection studies under controlled conditions have revealed that a high degree of humidity is necessary for the successful infection of *pan* plants by the fungus. These observations are in accord with the experience of Rolfs (1896) the original investigator of this fungus, of Peltier (1916) who worked with perennial garden plants in Illinois and of Bertus (1927) who worked with groundnut in Ceylon. A high temperature seemed also to be conducive to disease production and between 30° and 35° C. the fungus was most virulent infecting every plant with which it came into contact.

Field studies and observations have also borne this out. During the months of June and July when the disease causes the greatest damage the

prevailing temperature usually ranges from 30° to 35° C. and the relative humidity from 91 to 98.

(v) *Incubation period.*—Careful observations made during the inoculation experiments have revealed that the incubation period varies from 2 to 5 days depending on the tenderness of the tissue and the prevailing conditions of temperature and humidity. Signs of infection usually become visible on the second day, on the third day the plant usually begins to wilt and the plant usually dies in 4 to 5 days after inoculation.

V. Thermal Tolerance of Mycelium and Sclerotia

(i) *Mycelium.*—The thermal tolerance of the mycelium was found out by immersing 3 mm. blocks of mycelium collected from 3 days' old culture of the fungus at different temperatures ranging from 45°–55° C. for various lengths of time. Test-tubes containing 10 c.c. of sterile water were heated in a water-bath to the respective temperatures. Heating was done very carefully so that the temperature always remained constant and at the desired point. The mycelial blocks were dropped in the test-tubes containing the heated water. Immediately after the treatment cold sterile water was added and the temperature lowered down. The mycelia were then plated on oat agar and incubated at 28°–30° C. It was found that the mycelia were killed after three minutes' exposure at 50° and after two minutes' exposure at 52° C.

(ii) *Sclerotia.*—The thermal tolerance of the sclerotia was found out by immersing them for five minutes at different temperatures ranging from 50°–62° C. Test-tubes containing 10 c.c. of sterile water were heated in a water-bath to the respective temperatures. Heating was done very carefully so that the temperature always remained constant and at the desired point. Sclerotia selected from culture 30 days' old were dropped in the test-tubes containing the heated water. Immediately after the treatment cold sterile water was added and the temperature lowered down. The sclerotia were then plated on oat agar and incubated at 28°–30° C. It was found that all the sclerotia were killed after 5 minutes' exposure at 59° C. and by two minutes' exposure at 62° C.

It will thus appear that the sclerotia are more tolerant to heat than the mycelium.

VI. Viability of the Sclerotia

(i) *In the Laboratory.*—The fungus was grown on oat meal agar and a large number of sclerotia collected from a one month old culture and

stored on dry sterilized sand in cork tubes in the laboratory. Their viability was tested at frequent intervals by placing them on oat agar slants. As a result of a large number of tests it was found that seven months' old sclerotia gave 100 per cent. germination while those 8 and 10 months' old gave 15 and 20 per cent. germination respectively. All the sclerotia 16 months' old were found dead.

(ii) *At different depths in the soil.*—The viability of the sclerotia buried at different depths in the soil was studied. Sclerotia from one moth old culture were buried at 1, 2, 3, 4, 5 and 6 inches deep in ordinary *boroj* soils placed in glass cylinders. After 5, 15, 30, 45 and 60 days samples of sclerotia were recovered, surface sterilized and plated on oat agar. It was found that the sclerotia may be buried upto a depth of 4 inches in fairly moist soil for 60 days with only slightly affecting their viability, giving still 60 per cent. viable sclerotia. Those buried 5 inches deep for 45 and 60 days gave only 15 and 10 per cent. viable sclerotia respectively while those buried 6 inches deep lost their viability after 45 days.

VII. Perpetuation and Dissemination

(i) *Perpetuation.*—The fungus is essentially a soil dweller. It lives and multiplies in the soil where there is enough supply of organic matter and moisture, both of which are present in abundance in all types of *boroj* soils. The fungus may also survive in the infected plant tissue left in the soil. From a large number of these materials the fungus has been isolated. The fungus lives in the soil from year to year and from season to season by its sclerotia. The fungus remains in the sclerotial stage during seasons unfavourable to its growth. When conditions are favourable the sclerotia grow provided that the soil is sufficiently moist. The mycelium radiates from the sclerotium and creeps on the soil until it comes in contact with its host. Under field conditions the sclerotia are the chief source of infection.

(ii) *Dissemination.*—The disease occurs in the *pan boroj* soils where the fungus is present. In ploughing the soil that adheres to the implements, the feet of the animals and labourers may often carry the sclerotia of the fungus from field to field. The sclerotia are sufficiently light to be carried from one place to another by irrigation water. These bodies may also be carried by rain water from one plot to another and also through the earth that is used by the *baruis* every year for earthing up in the *borojes.*

The fungus may also be carried through planting setts but this does not happen usually. For, in selecting the setts for planting only the middle portion of the vines are chosen, the lower portion lying near the ground and

the top are usually discarded. The fungus can only be carried through setts when they are selected from portions that lie on or near the ground and are likely to harbour the sclerotia of the fungus on the surface. It is therefore necessary that the setts for planting be carefully selected.

VIII. Control of the Disease

The control of the parasite has proved particularly difficult owing to the fact that the fungus is a soil dweller and that it is not possible to sterilize the soil and kill the parasite by keeping the crop standing in the soil. When setts are planted in a soil already infested with the parasite the disease is bound to appear under favourable conditions and under such circumstances it becomes necessary to save the standing crop.

(i) *Fungicides.*—The following experiments were carried out for the last three years in the cultivarors' fields at Purangaon and at Kumrakapan and at the experimental plot at Sylhet for controlling the disease. *Borojes* indicating very severe outbreak of the disease were selected for treatments. The *borojes* were divided into randomised blocks and given the following treatments. Each treatment had six to eight replications.

The treatments were:

A. The plants were left untreated. They served as controls.

B. Plants were irrigated along the ridges with 2 : 2 : 50 Bordeaux mixture at the rate of one gallon per ridge of 8 ft.

C. Plants were irrigated along the ridges with kerol (1 in 1,000 parts of water) at the rate of one gallon per ridge of 8 ft.

D. Plants were irrigated along the ridges with phenyl (1 in 1,000 parts of water) at the rate of one gallon per ridge of 8 ft.

In each case treatments were commenced in early May, repeated at an interval of 30 days and stopped in October. Altogether there were five treatments, one treatment in each of the months May, June, July, August and September. The number of plants in each of the plots was carefully counted during the first treatment and before commencing the subsequent treatments the number of deaths in each of the plots was carefully noted. For economy of space the results obtained at Purangaon and Kumrakapan during the years 1941 and 1942 are summarised in Tables II and III.

It will be found from the results presented in Tables II and III that none of the treatments was effective in appreciably minimising the onslaught of the disease. The difference between the percentage of death in the treated and the control plots was not very marked, there was a high percentage of

TABLE II

Summary of the results of experiments done for controlling the disease at Purangaon and Kumrakapan in 1941

Treatments	Purangaon			Kumrakapan		
	Total No. of plants	Total No. of deaths	Percentage of death	Total No. of plants	Total No. of deaths	Percentage of death
A	420	112	26·6	517	129	24·9
B	427	102	23·8	502	104	20·7
C	412	77	18·6	528	96	18·1
D	421	96	22·7	498	97	19·4

TABLE III

Summary of the results of experiments done for controlling the disease at Purangaon and Kumrakapan in 1942

Treatments	Purangaon			Kumrakapan		
	Total No. of plants	Total No. of deaths	Percentage of death	Total No. of plants	Total No. of deaths	Percentage of death
A	725	174	24·0	679	129	18·9
B	730	143	19·5	682	102	14·9
C	719	119	16·5	672	81	12·05
D	727	126	17·3	680	89	13·08

death in all the treated plots. Similar results were obtained also at the experimental plots at Sylhet during the years 1942 and 1943. From these results it may safely be concluded that this disease cannot be effectively controlled by the use of fungicides.

(ii) *Earthing*.—It has been observed during the infection studies that when the sclerotia of the fungus were buried 3 inches or more in the soil no infection resulted. Rapid infection resulted when the sclerotia were on the surface or an inch deep in the soil. Exhaustive field observations revealed that in all cases of infection the sclerotia were lying either on the soil surface or about an inch or so below the surface soil. In some cases by actual counting 45 to 98 sclerotia were found lying on a square inch of surface soil, in the *boroj*. It was further observed that heavy rains during the season of occurrence of the disease help to bring the sclerotia to the surface and thus aid infection. Efforts were therefore made to control the disease by frequent earthing up of the ridges during the rains. Earthing up of the ridges

is a common cultural practice but it is usually done only after each manuring. During these trials it was done more frequently and every time after a heavy shower, the idea was to keep the ordinary surface soil covered with disease-free soil brought from elsewhere to a depth of 3 to 4 inches. This practice was given a wide trial and was found quite effective in reducing the percentage of death. Results of a number of experiments showed that where earthing up was carefully done there was either no death or the percentage of mortality varied from 2 to 7 per cent. while in the check plots it varied from 12 to 21 per cent.

IX. Prevention of the Disease

It has already been stated that the parasite survives in the soil and when setts are planted in infested *borojes* the disease appears under favourable conditions. It will, therefore, become quite clear that in order to prevent the disease the following methods should be given a trial:—

(*a*) Before planting the setts the infested soil should be sterilized. This can be done either by heat or by chemicals.

(*b*) After sterilizing the soil only carefully selected healthy setts should be planted.

(*c*) Subsequent infection through drainage water, soil and manure should be prevented.

(i) *Soil Sterilization.*—The following experiments were carried out to find the effect of soil sterilization on the prevention of the disease. A *boroj* where practically all the plants have died of the disease was selected. To this an additional dose of infective material was added by introducing cultures of the fungus grown on oat agar. The land was then divided into eight equal blocks and each block into five equal plots. There were five treatments and each treatment had eight replications. The treatments were as follows:

A. Soils left without any treatment. They served as control.

B. Soil was thoroughly ploughed to a depth of 8 inches. It was then burnt by flames thrown from a flame gun. The flame gun is designed to emit a flame of 2000° F. The burning was done very carefully and thoroughly. After the completion of the operation the temperature of the soil was taken and it was found to range from 75°–82° C. to a depth of six inches. During flaming the temperature would have been surely more than this. As this temperature is more than the thermal tolerance of the fungus it might safely be stated that during the burning the heat was sufficient enough to kill the fungus in the soil.

C. The soil was thoroughly ploughed and watered from a sprayer by a 2 per cent. solution of formaline (40 per cent.) so as to saturate the soil. The plot was then covered with wet sacks for 48 hours. The planting was done after 15 days when the smell of formaline has gone. The solution was applied at the rate of 3 gallons per suqare yard.

D. The soil was thoroughly ploughed and then saturated with a solution of kerol (1 in 600 parts of water). It was applied at the rate of three gallons per square yard.

E. Ground sulphur at the rate of 10 maunds per acre was added and mixed thoroughly with the first six inches of the soil.

Fifteen days after these treatments equal number of setts were planted in each of the plots. Only healthy setts were planted. Planting was done in August, 1942 and the plants kept under observation for two years. The number of deaths and the general growth and vigour of the plants were carefully noted every month. The number of deaths observed till August 1944 is recorded in Table IV.

TABLE IV

Effect of sterilization of disease infested soil on the incidence of the disease

Treatments	No. of plants in each block	Number of deaths in each block								Total No. of plants	Total No. of deaths	Percentage of death
		1	2	3	4	5	6	7	8			
A	60	11	9	13	16	19	14	11	15	480	108	22·7
B	60	..	1	..	..	..	2	..	..	480	3	0·6
C	60	2	2	..	4	1	2	..	1	480	12	2·5
D	60	1	..	..	3	2	..	4	1	480	11	2·3
E	60	5	3	1	2	5	1	2	..	480	19	3·9

It will be evident from the results presented in Table IV that the ravages of the disease can be appreciably minimised if the soil is sterilized with chemicals or heat before planting and only healthy setts planted thereafter. Heat has given the best results: formaline and kerol rank second and sulphur stands third.

In this connection it may be stated that soil sterilization by chemicals is a cumbrous method and requires considerable skill and intelligence on the part of the grower for its successful prosecution. As the cultivators are illiterate it can be safely presumed that it will not be practicable and the cultivators will not be able to carry this out effectively. Heat treatment,

however, will be a suitable method and the cultivators will be able to adopt this easily. It may be argued that on a field scale soil sterilization by heat or chemical is not practicable but in the cultivation of *pan* the method can be easily and profitably adopted. *Pan* is grown on very small plots of land varying from 1/16th to 1/18th of an acre and plots of such dimensions can be easily treated with heat.

In the absence of a flame gun the cultivator can very effectively sterilise his soil by burning it thoroughly with straw, thatching grass, etc. This has been tried by the author (1944) and has been found to yield very promising results.

(ii) *Deep Ploughing.*—Infection experiments have revealed that the parasite loses its infective power when its sclerotia are buried three inches or more in the soil. This fact has suggested the possibility of preventing the disease by deep ploughing whereby it is possible to bury the fungus to a depth of three or more inches. The method has been given an exhaustive and careful trial and it has been found to minimise considerably the incidence of the disease. In the deeply ploughed plots the percentage of death has been found to range from 2 to 5 while in the control plots it varied from 12 to 21·5 per cent. It may be remarked in this connection that by deep ploughing it is not possible to bury all the sclerotia beyond three inches. Ploughing, cross-ploughing and laddering might bring some of the sclerotia at the surface or an inch or two below the surface. But in spite of this limitation the method is a suitable and effective one and the cultivators can adopt this with profit. The efficiency of this method can be greatly increased by frequently piling in disease-free earth along the ridges, thus helping the burial of the sclerotia which might have come to the surface beyond a depth of three inches.

(iii) *Rotation.*—It has been found that under laboratory conditions the sclerotia lose their viability after 16 months while under field conditions the period is less. From this it might appear that rotation may be heplful in preventing this disease. Field observations for the last four and half years over the area where the disease occurs have demonstrated that fallowing of the *boroj* land or growing of a different crop in it for three years has not eliminated the disease. There appears to be two probable reasons for this: the fungus may during the absence of its host live a saprophytic life in the soil or as the crops that are commonly grown in *boroj* lands are *til* (*Sesamum orientale*), *mug* (*Phaseolus radiatus*), brinjal, chillies and tomato, all of which are susceptible to the attack of this parasite, the fungus survives in its new hosts. Field observations have actually brought to notice instances

where tomatoes, chillies, brinjals, *til* and *mug* grown on *boroj* land which previously bore an affected *pan* crop, also showed symptoms of the disease. As the fungus has a wide range of hosts it may safely be stated that it will not be a suitable and effective method for fighting this menace.

From a consideration of the methods outlined above it will appear that in order to get a healthy crop the cultivators will have to adopt the following measures:—

(i) The soil of the infested field should be sterilized before planting. This can be done either by heat or chemicals. Heat, *i.e.*, burning of the soil by straw and thatching grass will be found very suitable and effective.

(ii) After the sterilization of the soil only carefully selected healthy setts should be planted.

(iii) If perchance a plant or plants are found attacked, such affected plant or plants should be immediately removed along with the sclerotia lying near the base of the plant in the soil and completely destroyed by burning.

(iv) Subsequent infection through drainage water, manure or earth that is brought from outside for earthing up should be guarded against.

(v) During rains the sclerotia that come up to the surface should be carefully picked up and destroyed or covered with a thick layer of disease-free soil in order to destroy their infective ability.

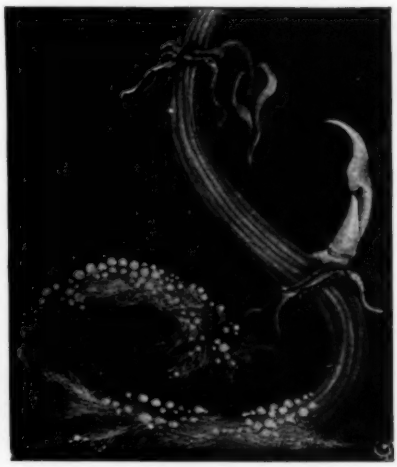
X. Summary

Sclerotial wilt of *pan* (*Piper betle*, Lin.) due to *Sclerotium rolfsi* Sacc. is a serious disease and occurs in villages Purangaon, Phulbari, Faua, Erangabaj and Kumrakapan. The percentage of mortality has been found to vary from 4 to 31 in the different villages.

The syptoms of the disease have been described.

The incidence of the disease has been studied and it has been found that the disease appears during the middle or later part of May and continues to be vigorous during the later part of June and whole of July. From the first or second week of August the rate of death of plants gradually falls off and the disease ceases by the first week of September.

Inoculation experiments have been carried out with the pure culture of the fungus and its pathogenicity established. It has been found that the sclerotia fail to infect the plants if they are buried three or more inches deep in the soil. Infection is rapid when the sclerotia lie on the surface or an inch below. Infection is delayed when they are buried two inches deep.



(b)

Symptom of the disease : (*a*) A wilted *pan* plant, (*b*) Lower portion of a wilted plant showing the mycelial growth and sclerotial formation on the host and soil

It has been found that a high degree of humidity is necessary for the successful infection of *pan* plants by the parasite. The incubation period has been found to vary from 2 to 5 days.

It was found that the mycelia were killed after three minutes' exposure at 50° C. and after two minutes' exposure at 52° C. The sclerotia were killed after 5 minutes' exposure at 59° C. and by two minutes' exposure at 62° C.

It was found that under laboratory conditions the sclerotia retained their viability for 12 months, after 16 months all the sclerotia were dead. Under field conditions the sclerotia may be buried up to a depth of four inches in fairly moist soil for 60 days with only slightly affecting their viability. All sclerotia buried six inches deep in the soil lost their viability after 45 days.

The disease is perpetuated and disseminated through its sclerotia. Implements, feet of the animals and labourers, drainage water, manure and earth help the spread of the sclerotia.

Control methods have been studied. Irrigation of the plants along the ridges with Bordeaux mixture, kerol and phenyl has not been found effective in appreciably minimising the incidence of death. Earthing up with a view to cover the sclerotia to a depth of more than three inches has been found successful in decreasing the mortality.

In order to prevent the disease it has been found necessary to sterilize the soil either by heat or by chemicals and then to plant only healthy setts. Sterilization of the soil by heat has been found to be the most suitable and effective method. Deep ploughing has been found helpful in minimising the incidence of the disease.

Rotation has not been found effective in preventing the disease.

Acknowledgments

My grateful thanks are due to Prof. H. Chaudhuri, D.Sc. (Lond.), Head of the Department of University Teaching in Botany, and Director, Kashyap Research Laboratory, Panjab University, for critically going through the manuscript and valuable suggestions.

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DISEASES OF *PAN* (*PIPER BETLE*) IN SYLHET, ASSAM

Part VI. *Gloeosporium* Leaf-Spot

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(With one Plate)

Received April 11, 1945

(Communicated by Prof. H. Chaudhuri, D.Sc., F.A.Sc.)

I. Introduction

A leaf-spot of *pan* (*Piper betle*, Linn.) due to *Glœosporium* sp. has been found widespread and occurs throughout the *pan*-growing tracts. A few leaves here and there in a *boroj* are usually attacked. The number of spots on a leaf commonly varies from one to four, rarely the entire surface of a leaf is covered with a large number of spots. It is when leaves are covered with a large number of spots that they lose their market value, otherwise they pass as normal leaves. In general, the disease is of minor importance and the damage done is slight.

II. Symptoms of the Disease

The disease starts on any portion of the leaf. Usually younger leaves are more susceptible to infection than old ones. When young the spots are small, circular or irregular and brownish in colour, when old they increase slightly in size, become more irregular and the colour darkens slightly approaching straw colour. The spots are always somewhat sunken. In some cases the surface of the lesion is slightly roughened by acervuli that have broken through the epidermis. In certain instances the central dead portions of the spots have been noted to fall off. Plate XIII, shows the symp toms of the disease.

III. Etiology

A large number of isolations were made from diseased specimens collected from the different *pan*-growing tracts. A species of *Glœosporium* was always obtained in culture. The pathogenecity of the fungus was established in the usual manner by isolation, culture inoculation and comparison.

The morphological characteristics of the fungus were studied in culture. When grown on oat agar this fungus produces a minimum of mycelium and large ochraceous salmon-colour spore masses. The spores are unicellular, hyaline, straight, often taper slightly at both ends and measure 8·4–19μ $\times$ 3–5–6·4μ (average 13·4 $\times$ 4·6μ) which is well within the range given by Shear and Wood (1913) for the conidia of *Glomerella cingulata* (Stonem.) Spauld and v. Schrenk. As such the present fungus is identified as the conidial stage of *G. cingulata.*

IV. Perpetuation and Dissemination of the Pathogene

(i) *Perpetuation.*—Species of *Glomerella* are known to be weakly parasitic in their imperfect stages. They are cosmopolitan organisms and may be found on a wide range of weak and dead plants. A careful investigation has revealed that the fungus survives on the dead host tissues lying in piles along the ridges and the dead leaves and vines left in the plantations.

(ii) *Dissemination.*—Agati (1921) showed that the conidia of *Glœosporium musarum* Cke. and Massee on banana are disseminated by wind and by insects. Higgins (1913) reported that wind helps in the dissemination of the spores of *Glœosporium* in the field. According to Edgerton (1908) conidia of *G. nervisequum* cannot be blown away by the wind because they are held firmly by mucilaginous substance. He believes that spore dissemination is affected by rain. Clinton (1902) states that the conidia of *G. fructigenum* are disseminated by small pomaceous flies of the genus *Drosophila.*

The methods of dissemination of the spores of the fungus were studied, and it was observed that the spores can be disseminated by wind, rain and water passing over the lesions.

V. Control Measures

The disease has been found rarely serious requiring any methods of control. When serious it can be easily controlled by spraying the plants with a 2:2:50 Bordeaux mixture or Perenox. Both these mixtures have been given a wide trial during the last three years and have been found to yield very satisfactory results. In addition to spraying clean cultivation and sanitary methods should be followed. The dead vines and leaves should be collected and destroyed and not allowed to rot in the *boroj.*

VI. Summary

A leaf-spot of *pan* (*Piper betle* Lin.) has been found widespread and occurs throughout the *pan*-growing tracts.

The symptoms of the disease have been described.

The causal organism has been identified to be the conidial stage of the fungus *Glomerella cingulata* (Stonem.) Spauld. and V. Schrenk and its pathogenicity has been established.

The fungus is disseminated by wind, rain and water passing over the lesions and survives on dead vines and leaves lying in the plantations.

The disease can be controlled by clean cultivation, destruction of dead leaves and vines and the spraying of the plants by 2:2:50 Bordeaux mixture or Perenox.

Acknowledgments

My grateful thanks are due to Prof. H. Chaudhuri, D.Sc. (Lond.), Head of the Department of University Teaching in Botany, and Director, Kashyap Research Laboratory, Panjab University, for critically going through the manuscript.

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Symptoms of the disease

THE NATURAL OCCURRENCE OF ERGOT IN SOUTH INDIA—II

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Received June 11, 1945
(Communicated by Rao Bahadur Sri. V. Ramanatha Ayyar, F.A.SC.)

In an earlier communication on the natural occurrence of ergot in South India, the authors (Thomas *et al.*, 1945) had recorded a number of new hosts of *Claviceps* observed for the first time in South India. Since then three more of the common grasses have been found to be naturally infected by *Claviceps* and the descriptions of the fungi are given below.

1. *Panicum maximum.*—This is the common cultivated 'Guinea grass' grown as a fodder. Shepherd (1926) has observed a *Sphacelia* on this host in Mauritius. But he has not given any description of the fungus or its spores. Barger (1931) has mentioned that inoculations of *C. microcephala* on *Panicum maximum* gave negative results. Apart from these the authors have not come across any reference of *Claviceps* on this host.

In December 1944, there was heavy infection in a plot of *Panicum maximum* by *Claviceps* at the Central Agricultural Research Station, Coimbatore. Nearly 30 per cent. of the spikelets were involved. But due to easy shedding many ergotised spikelets had fallen and only a few were seen at a time on the panicles. The conidial and sclerotial stages were present. The honeydew is a creamy semi-solid mass protruding beyond and overflowing the lemma and the palea. The spores are hyaline, oblong or reniform with rounded ends, measuring $18{\cdot}9 \times 6{\cdot}0\ \mu$ ($13{\cdot}8$ to $24{\cdot}2 \times 3{\cdot}3$ to $8{\cdot}6$). The sclerotia are prominent and are firmly clasped by the glumes at the base. The whole sclerotium with the glumes falls off easily from the panicle. The sclerotia are greyish white with the apex much lighter (cream coloured), tapering and slightly bent. They measure 5 to $6 \times 0{\cdot}75$ to 1 mm. (Plate XIV, Fig. 1). The conidial characters suggest that this fungus may be similar to the *Claviceps* on *Cynodon dactylon*. Further studies to elucidate this are in progress.

2. *Themeda cymbaria* is a common tall-growing wild grass on the Pulneys in the neighbourhood of Kodaikanal, with big panicles. In December 1944 this grass was observed to be infected by *Claviceps* over a wide area at Kodaikanal. The honeydew is not very conspicuous and the presence of the pink overgrowth of a *Fusarium* was always helpful in locating infected panicles. Closer examination revealed inconspicuous whitish conidial masses in some of the spikelets. The spores are small, oblong or oval, hya-

line and measure 4·1 × 1·7 μ (3·4 to 5·2 ×1·7). The sclerotia are of varying lengths, 3·5 to 10·0 × 0·5 to 0·7 mm. often very conspicuously projecting out of the spikelets (Plate XIV, Fig. 2). They are slightly curved, dark grey in colour, with a number of grooves and wrinkles on the surface. The base of the sclerotium is often of a violet colour. It may be mentioned in this connection that the violet colour at the base of the sclerotium is common in *Claviceps* on a number of grasses. From the conidial and sclerotial characters, this fungus is found to closely resemble the *Claviceps* on *Amphilophis Foulkesii*.

3. *Digitaria Wallichiana*.—The infection on this host was prevalent both at Kodaikanal and at Ootacamund from November to February. The conidial stage occurs as a whitish mass visible between the gaping glumes. The conidia are hyaline, oblong, sometimes curved and measure 16·6 × 4·5 μ (10·4 to 20·7 × 3·5 to 5·2). The sclerotia are small, smooth, dark brown and measure 1·5 to 3 × 1 mm. (Plate XIV, Fig. 3). They are oval to oblong with an abruptly flattened apex from the middle of which a short projection is formed. Though held between the gaping glumes the sclerotia easily separate from the spikelet. The conidial measurements and the appearance of the sclerotia suggest a close resemblance to *Claviceps* occurring on *D. chinensis*. Hansford (1941) has recorded *Claviceps digitariæ* on *Digitaria scalaris* from Africa.

The authors had recorded *Claviceps viridis* on *Oplismenus compositus* from the higher elevations of Nilgiris and Kodaikanal. In January 1945 the same fungus was found on this host at Yercaud on the Shevaroys (5,000 feet above mean sea-level).

The fungi observed on these new hosts can be classified into one or other of the 5 groups into which *Claviceps* occurring on the 18 hosts were classified in the earlier communication (Thomas *et al.*, 1945).

Summary

Ergot was observed on *Panicum maximum* at Coimbatore, on *Themeda cymbaria* at Kodaikanal and on *Digitaria Wallichiana* at Kodaikanal and at Ootacamund. The conidial and sclerotial measurements are given. These are new records of hosts for *Claviceps*. *Claviceps viridis* on *Oplismenus compositus* was recorded from Yercaud also.

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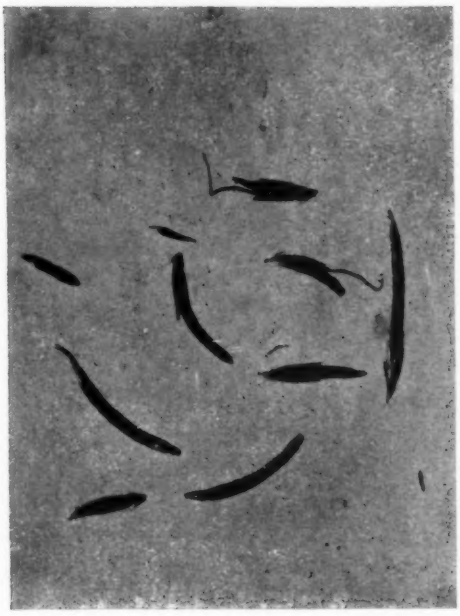

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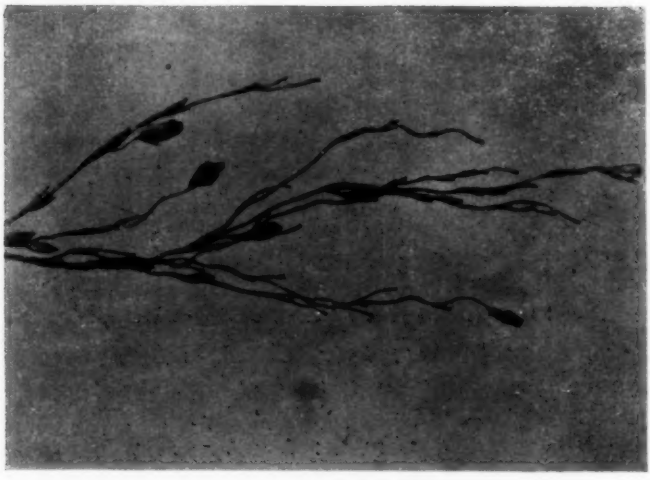

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FIG. 1. Infected panicle of *Panicum maximum* showing ergots. × 2.
FIG. 2. Ergots from *Themeda cymbaria*. × 2.
FIG. 3. Panicle of *Digitaria Wallichiana* showing two ergots. × 2.

CYTOLOGICAL STUDIES IN PALMÆ

Part I. Chromosome Numbers in a Few Species of Palms of British India and Ceylon

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(With 30 Figures in the Text)

Received May 15, 1945

(Communicated by P. Maheshwari, D.Sc., F.N.I., F.A.Sc.)

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I. Introduction

About eight years ago Tischler (1937) drew the attention of Indian Botanists to the existence of 'great gaps' in our knowledge of chromosome numbers in a good many tropical and sub-tropical families of Angiosperms. The author in collaboration with Raghavan (1943) recorded the chromosome numbers for 7 genera and 24 species of the family Zingiberaceæ. The tropical family Bignoniaceæ formed the subject-matter for another investigation and chromosome numbers have been determined for 43 species spread over 18 genera (Venkatasubban, 1945). The present investigation, too, has been made with a view to provide similar information regarding the essentially tropical family Palmæ.

The family Palmæ includes 131 genera and about 1,100 species (Blatter, 1926). Of these about a hundred species may said to be indigenous to India and Ceylon but very few of them have received the attention of the cytologist. Santos (1928) has recorded the haploid chromosome number for *Cocos nucifera* as $n = 16$ and also given details of meiosis for this species.

In *Trachycarpus excelsus* Sinotô (1929) found one unequal pair of chromosomes, in addition to 17 autosomic pairs, in the first meiotic division of the microspore mother cells. Gaiser (1930) and Tischler (1938) have published chromosome numbers for 15 species of the family. The following will sum up the chromosome numbers, so far known, in the family:—

Genus and Species	*n*	2*n*	Author, Year, etc.
Pritchardia filamentosa ..		24	Nemec, 1910
Chamædorea corallina Karst. ..	12–14		Söderberg, 1919
C. glaucophylla	13		Süssenguth, 1920
C. Sartorii	6–8		,, 1920
C. Karwinskiana		26	,, 1921
Nipa fruticans	8		Radermacher, 1925
Cocos nucifera	16		Santos, 1928
Trachycarpus excelsus Wendl. var. Fortunei Mak.	17+X 17+Y		Sinotô, 1928; in the heterotypic division of microspore mother cells
Phœnix canariensis	18		
P. dactylifora	18		
P. dactylifera		28	Nemec, 1910
P. farinifera	18		
P. Hanceana	18		
P. humilis	18		
P. reclinata	18		
P. sylvestris	18		
Sabal causiarum Becc. ..		36	Bowden, 1945
Sabal minor Jacq.		36	,, 1945
Sabal Palmetto Lodd		36	,, 1945

In the present investigation, the diploid chromosome numbers have been determined for 30 species spread over 23 genera. An idiogram study has also been attempted for some of the species. No attempt has been made however, to study these plants with reference to sex chromosomes, as only the root-tips of young seedlings were available for investigation and as such it is not possible even to say whether the chromosomes counted and drawn belong to the male plant or the female one in the case of the dioecious species. Nor could any attempt at a critical analysis of the chromosome morphology be made since the main object was to record the chromosome numbers and to fill up the gaps to which our attention has been drawn nearly 8 years ago by Prof. Tischler.

II. Materials and Methods

Materials for the present study were obtained from the Soundarya Nursery, Madras, in the form of seedlings. The root-tips were fixed between 10–11 A.M., on warm sunny days, after the removal of the sheath which usually occurs around them; this was found to be quite necessary, as otherwise, fixation was unsatisfactory and the metaphase chromosomes

presented a clumped appearance. Its presence also caused some difficulty in sectioning. Other technical details were similar to those adopted for the Bignoniaceæ (Venkatasubban, 1945). The drawings were made with a camera lucida at an approximate magnification of 5200 diameters, and reduced to half in reproduction.

III. Observations—Somatic Chromosomes

The following somatic chromosome numbers were counted from root-tip mitoses:—

		2*n*
	CORYPHINÆ	
1.	*Phœnix paludosa* Roxb.	36
2.	*Rhapis flabelliformis* L.F.	36
3.	*Thrinax excelsa* Lodd, Griseb.	36
4.	*Thrinax argentea* Lodd	36
5.	*Licuala peltata* Roxb.	28
6.	*Livistonia chinensis* R.Br.	36
7.	*Livistonia rotundifolia* Mart.	36
8.	*Pritchardia pacifica* Seem et H. Wendl.	36
9.	*Pritchardia filifera* Linden	36
10.	*Pritchardia granais* Hort.	16
11.	*Sabal adansoni* Guers.	36
12.	*Sabal umbracaulifera* Mart.	36
	BORASSINÆ	
13.	*Latania rubra* Jacq.	32
14.	*Borassus flabellifer* Linn.	36
	LEPIDOCARYINÆ	
15.	*Calamus* sp. (unidentified)	28
	CEROXYLINÆ	
16.	*Arenga saccharifera* Labill.	32
17.	*Dypsis madagascariensis* Hort.	36
18.	*Oreodoxa regia* Kth.	36
19.	*Howea forsteriana* Becc.	32
20.	*Heterospatha elata* Scheff.	32
21.	*Nephrosperma van houtteana* Wendll.	32
22.	*Oncosperma filamentosum* Bl.	32
23.	*Kentia Macarthurii* Hort.	32
24.	*Kentia Sanderiana* Hort.	32
25.	*Dictyosperma album* Wendl.	32
26.	*Areca catechu* Linn.	32
27.	*Areca lutescens* Bory.	32
28.	*Cocos schizophylla* Mart.	32
29.	*Martinezia caryotæfolia* Humb. & Kth.	32
30.	*Elæis guineensis* Jacq.	32

Coryphinæ

Phœnix paludosa.—The somatic chromosomes, as observed during metaphase, in the root-tip mitosis are represented in Fig. 1. Thirty-six chromosomes were counted, showing either sub-median or sub-terminal constrictions, the former being the case with the long chromosomes in the complement. The chromosomes did not exhibit much difference in their

lengths excepting some six which were definitely smaller. The chromosomes of this species range in size between 0·2 μ–0·08 μ.

Rhapis flabelliformis.—Thirty-six chromosomes (Fig. 2 A) were counted during metaphase in the root-tip cells. The chromosomes have been resolved into their homologues and are represented in Fig. 2 B. As in the previous case, most of the chromosomes show sub-median constrictions; a few, however, have sub-terminal constrictions. The chromosome complement consisted of 2 long (0·4 μ–0·34 μ), 11 medium-sized (0·3 μ–0·2 μ) and 3 short pairs (0·16 μ–0·14 μ).

TEXT-FIGS. 1—10 B

Thrinax excelsa.—Fig. 3 A represents a somatic plate of this species and Fig. 3 B is idiogram of the same. 36 chromosomes were counted. The chromosomes do not show any departure in their morphology as compared to the cases described so far. However, there is one striking difference namely, that a few of the chromosomes of this species are the longest in the 30 species investigated by me. A reference to Fig. 3 B will show that there

are 2 long (0·7 μ–0·54 μ), 11 medium (0·44 μ–0·22 μ) and three short pairs of chromosomes (0·18 μ–0·12 μ).

Thrinax argentea.—This species exhibited the same dipliod chromosome number of $2n = 36$ as *T. excelsa* (Fig. 4 A). From the idiogram (Fig. 4 B), it can be seen that there are 2 long (0·52 μ–0·40 μ), 12 medium (0·30 μ–0·24 μ) and 2 short (0·16 μ) pairs of chromosomes. The chromosome morphology is thus quite similar to that of *T. excelsa.*

Licuala peltata.—The somatic chromosomes of this species (Fig. 5) are quite distinct, both in number and morphology, from those described so far. This is the only instance, among the investigated species, under the tribe Coryphinæ, where a diploid number of $2n = 28$ was counted. The chromosomes were mostly short and thick. Excepting some 4 numbers, which may be called long and which have median constrictions, the rest were shaped like tiny rods in which the constrictions could not be made out at all. The former had an average length of 0·14 μ while the smaller measured about 0·8 μ

Livistonia chinensis.—The diploid chromosome complement of this species is represented in Fig. 6 and that of *L. rotundifolia* in Fig. 7. Both the species agree in having the same chromosome number ($2n = 36$). However, the longest chromosome of *Livistonia chinensis* measured 0·22 μ while that of *L. rotundifolia* was only 0·16 μ. But for this slight difference in size, the chromosome complements of the two species are quite similar to each other.

Pritchardia.—The somatic chromosomes of the three species examined—*P. pacifica* (Fig. 8), *P. filifera* (Fig. 9) and *P. grandis* (Fig. 10 A) are typically small. *P. pacifica* and *P. filifera* have the same number, $2n = 36$ and they also agree in their morphology. The longest and the shortest chromosomes in *P. pacifica* measured 0·18 μ and 0·06 μ, respectively, and this has been found to be approximately true for *P. filifera* also. In the latter species one of the chromosomes has been found to carry a trabant. In *P. grandis* the chromosomes are more condensed and therefore conspicuous although they have the same length as those of the other two species. Apart from this difference, it was found that the diploid number for this species is only 16 compared to 36 for the other two species of the same genus. Fig. 10 B shows the chromosomes of *P. grandis* arranged according to their lengths. The longest chromosome measured about 0·2 μ and the shortest 0·12 μ.

Sabal.—The two species of *Sabal*—*S. adansoni* (Fig. 11) and *S. umbraculifera* (Fig. 12), although agreeing in their chromosome numbers ($2n = 36$) show some difference in their morphology. Those of *S. adansoni*

were found to be bigger in size compared to *S. umbracaulifera*. For instance, in *S. adansoni* (Fig. 11) there are the two characteristic pairs of long chromosomes AA′ and BB′ with a length of 0·34 μ–0·28 μ. In *S. umbracaulifera* also there are two long pairs of chromosomes, but these are only 0·24 μ long. Further, the two long pairs in *S. adansoni* exhibited sub-median

TEXT-FIGS. 11—19 B

constrictions while in *S. umbracaulifera* these showed sub-terminal constrictions. The shrotest chromosome in *S. adansoni* measured 0·1 μ while that of *S. umbracaulifera* measured only 0·8 μ.

Borassinæ

In the tribe *Borassinæ*, only two species, *Latania rubra* and *Borassus flabellifer* were available for study. *Latania rubra* (Fig. 13) revealed a diploid number of $2n = 32$ chromosomes. The biggest chromosome in the complement measured about 0·34 μ and the smallest about 0·1 μ. The majority of the chromosomes exhibited sub-terminal constrictions.

Borassus flabellifer (Fig. 14) like the majority of the species investigated showed a diploid number of $2n = 36$ chromosomes. The chromosomes were quite large; the biggest of the lot measured about $0{\cdot}48\,\mu$ and the shortest about $0{\cdot}2\,\mu$. They also exhibited either sub-median or sub-terminal constrictions.

Lepidocaryinæ

Only one species belonging to this tribe was available for study, a *Calamus* growing wild round-about Annamalainagar (South India). However, the specimen could not be determined exactly. The somatic complement of this unidentified species was found to be $2n = 28$ (Fig. 15 A). The chromosomes are arranged according to their lengths and shown in Fig. 15 B. The longest member in the complement measured $0{\cdot}26\,\mu$ and the shortest $0{\cdot}14\,\mu$.

Ceroxylinæ

Fifteen different species of the tribe *Ceroxylinæ* were examined and it has been found that the chromosome number in 13 out of the 15 species is $2n = 32$ while the remaining two species have a diploid number of $2n = 36$.

Arenga saccharifera.—The somatic chromosomes of this species (Fig. 16 A) were found to fall into three groups, on the basis of their lengths, namely, long, medium and short. From Fig. 16 B, it can be seen that there are 4 pairs of long chromosomes ($0{\cdot}46\,\mu$–$0{\cdot}32\,\mu$) with submedian constrictions, ten pairs of medium-sized chromosomes ($0{\cdot}28\,\mu$–$0{\cdot}2\,\mu$) and two pairs of short chromosomes ($0{\cdot}16\,\mu$–$0{\cdot}14\,\mu$).

Dypsis madagascariensis.—Fig. 17 A represents the polar view of a somatic metaphase plate in a root-cell in which 36 chromosomes can be counted. Fig. 17 B represents the diploid set, of this species, arranged according to their lengths. The chromosome pairs form a graded series without any wide gaps. Some three pairs are definitely longer and have a length ranging between $0{\cdot}26\,\mu$–$0{\cdot}22\,\mu$. The shortest member in the complement measured about $0{\cdot}1\,\mu$.

Oreodaxa regia.—This species revealed the presence of 36 chromosomes which are represented in Figs. 18 A and 18 B. There are two long pairs ($0{\cdot}34\,\mu$–$0{\cdot}3\,\mu$), 15 medium ($0{\cdot}24\,\mu$–$0{\cdot}16\,\mu$) and one short ($0{\cdot}1\,\mu$).

Howea forsteriana.—Figs. 19 A and 19 B represent the somatic complement ($2n = 32$) of this species. As seen in Fig. 19 B there are two pairs of long chromosomes, with median constrictions and an average length of about $0{\cdot}34\,\mu$; 12 pairs of medium chromosomes ($0{\cdot}28\,\mu$–$0{\cdot}18\,\mu$) and two pairs of short chromosomes ($0{\cdot}16\,\mu$).

Heterospatha elata.—The somatic chromosomes of this species ($2n = 32$) are shown in Figs. 20 A and 20 B. There are 4 pairs of long chromosomes ($0{\cdot}44\,\mu$-$0{\cdot}38\,\mu$), two of which show median constrictions while the other two sub-median. The 9 pairs of medium chromosomes were found to range from $0{\cdot}32\,\mu$ and $0{\cdot}18\,\mu$ in length. The three short pairs have an average length of $0{\cdot}16\,\mu$, *i.e.*, they are only slightly smaller than the smallest of the medium-chromosome. The chromosome complement of this species is morphologically quite comparable to those of the *Thrinax* sp., or *Raphis flabelliformis*.

TEXT-FIGS. 20 A—27 B

Nephrosperma van houtteana.—The somatic complement of this species consists of 16 pairs ($2n = 32$) of graded chromosomes (Fig. 21 A, 21 B). These present a striking resemblance to those of *Dictyosperma album* which also shows a diploid number of $2n = 32$ chromosomes. The chromosomes of *Nephrosperma van houtteana* appear to possess, mostly, sub-terminal

constrictions. The longest chromosome within the lot measured about $0{\cdot}34\,\mu$ and the shortest $0{\cdot}1\,\mu$.

Oncosperma filamentosum.—Fig. 22 A represents the polar view of a metaphase plate in a root-cell of this species showing 32 chromosomes. These have been resolved into their homologues and represented in Fig. 22 B.

Kentia Macarthurii.—(Fig. 23 A) and *K. Sanderiana* (Fig. 24 A) each show a diploid number of $2n = 32$ chromosomes. Figs. 23 B and 24 B respectively, represent the chromosomes of these two species arranged according to their lengths. In both, 4 pairs of long chromosomes, indicated by the letters l_1, l_2, l_3, and l_4 (Figs. 23 B and 24 B), can easily be made out. The 9 pairs of medium chromosomes and 3 pairs of short chromosomes are also common to both. The short chromosomes have been marked by the letters S_1, S_2, and S_3 (Figs. 23 B and 24 B).

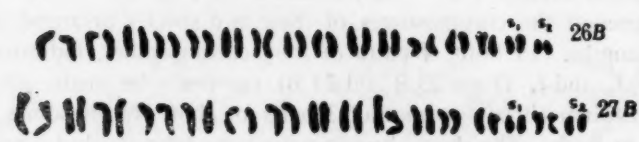
Dictyosperma album.—This species has a diploid number of 32 chromosomes (Figs. 25 A and 25 B) which bear a close resemblance, both in number and morphology, to those of *Nephrosperma van houtteana.* There are 4 long, 10 medium and 2 short pairs.

Areca lutescens.—Fig. 26 A represents a metaphase plate, in polar view, showing 32 chromosomes. The idiogram is shown in Fig. 26 B. The long chromosomes merge imperceptibly into the medium ones, so much so, it is rather difficult to distinguish the long chromosomes from the medium ones. However, there was no difficulty in the recognition of the short chromosome pairs S_1, and S_2 (Fig. 26 B).

Areca catechu.—The chromosome complement of this species, in the root-cell (Figs 27 A and 27 B) is essentially similar to that of *A. lutescens* but for a silght difference in size. The chromosomes of *Areca catechu* are slightly bigger than those of *A. lutescens.* Here again, two pairs of short chromosomes—S_1 and S_2 (Fig. 27 B), could be made out as in *A. lutescens.* The diploid chromosome number for both the species has been found to be $2n = 32$.

Cocos schizophylla.—Fig. 28 shows the $2n = 32$ chromosomes, which represent the somatic complement of this species. They exhibit prominent median or sub-median constrictions. The constituents of the chromosome complement show variation in length from $0{\cdot}32\,\mu$ to $0{\cdot}12\,\mu$.

Martinezia caryotæfolia.—Fig. 29 represents the 32 somatic chromosomes of this species. These bear a close resemblance to those of *Cocos schizophylla.* The longest member in the complement measured $0{\cdot}34\,\mu$ and the shortest $0{\cdot}18\,\mu$.



TEXT-FIGS. 28—30

Elæis guineensis.—The somatic complement of this species (Fig. 30) has been found to consist of 32 chromosomes. They are quite small in size with one very short pair.

IV. DISCUSSION

(*a*) *Chromosomal and Base Numbers*.—Chromosomal numbers are now available for 48 species of *Palmæ*. But, considering the total number of genera and species in this family (131 genera and 1,100 species) it may still seem premature to put forward any serious conclusions either on their phylogeny or on the probable base numbers. For, Babcock (1934) has already warned us against the danger:—"A mere study of the distribution of chromosome numbers in a small fraction of the species in a family or genus and especially without due consideration of other evidence on taxonomic relationships in those groups may lead to quite erroneous conclusions concerning basic chromosome numbers". Nevertheless a few remarks, of a general nature, may not be out of place.

The most frequent number is $2n = 36$. This has been found true for all the nine species of *Phœnix*, investigated so far, 12 out of the 14 species coming under the tribe *Coryphinæ*, *Borassus flabellifer* and two species of the tribe *Ceroxylines—Dypsis madagascariensis* and *Oreodoxa regia*. Thus out of a total of 48 species as many as 24 exhibit a diploid chromosome number of 36.

The next important number, which is also equally dominant, is $2n = 32$. This number is encountered mostly among the members of the tribe *Ceroxylinæ*. Out of a total of 20 species, 13 species show this number. *Latania rubra*, a member of the tribe *Borassinæ* also exhibits this number.

It is conspicuous by its absence in the 24 investigated species of the tribe *Coryphinæ*.

Other prevailing numbers, in the family, are $2n = 28$, 26, 24 and 16. A diploid number of 28 chromosomes is found in the unidentified species of *Calamus* (Lepidocaryinæ) and *Licuala peltata* (*Coryphinæ*). The number, $2n = 26$ has been recorded for *Chamædorea glaucophylla* and *C. Karwinskiana* of the tribe *Ceroxylinæ* (Süssenguth, 1920, 1921). A $2n$ number of 24 chromosomes has been counted for *Pritchardia filamentosa*. From the available data, it can be seen that, there are 2 instances where the number 16 ($2n$) occurs; one in *Pritchardia grandis* (*Coryphinæ*) and the other in *Nipa fruticans* of the tribe *Phytelephantinæ* (Radermacher, 1925). Though the chromosome numbers 28, 26, 24 and 16 are found in the different species of *Palmæ*, they are not as frequent as the numbers 16 and 18.

These chromosomal numbers would suggest that the different species of palms have been evolved along three principal lines. One line of evolution from a stock of $2n = 16$ as the base number; the second from a base number $2n = 18$ and a third from a base number $2n = 14$. At present the number $2n = 18$ is merely a hypothetical figure, being so far unrecorded for any of the *Palmæ*. However, the base numbers $2n = 16$ and $2n = 14$ have actually been met with, respectively, in *Pritchardia grandis* and *Chamædorea Sartorii*.—The next question is whether 16 is the primary base number from which the others were derived, or whether the numbers $2n = 14$ and $2n = 18$ came into existence quite independently and are also to be regarded as primary.

It appears more likely, however, that the numbers 14 and 18 were secondarily derived from 16. The process can be visualized as fallows:— If 4 of the chromosomes, in the ancestral type with a complement of 16, were to fuse in pairs, the result will be a form with only 14 chromosomes. In this 2 of the chromosomes will be bigger than the rest. If duplication of this set with 14 chromosomes were to take place, there will result forms with 28 chromosomes. Such forms will, no doubt, contain 4 big chromosomes. *Licuala peltata*, a 28-chromosomed species does show 4 big chromosomes as can be seen from Fig. 5. In the idiogram of the Rattan species (Fig. 15 B) also, another of the 28-chromosomed type, two pairs of long chromosomes AA′ and BB′ can be observed.

Thus fusion seems to have played a part, in the establishment of a secondary basic number in the members of this family. Similar instances of an unmistakable nature have also been observed in the members belonging to other Angiospermic families. For example, the genesis of the longest

pair of chromosomes with sub-median constrictions in *Crocus ochroleucus* has been explained as due to fusion of smaller chromosomes (Pathak, 1940). Davie (1933) has observed and recorded similar instances for some of the members of the Malvaceæ. *Cardamine pratensis* (Lawrence, 1931), *Fritillaria ruthenica* and *Stenobothrus* (Darlington, 1937), and others are similar instances.

The derivation of a secondary base number of a higher magnitude from a primary base number (in this case the derivation of $2n = 18$ from $2n = 16$) can be explained on the basis of fragmentation. If two of the chromosomes were to fragment in the 16-chromosomed ancestor, there would be produced a new form with 18 chromosomes of which 4 would be particularly short. If polyploidy comes into play, as it undoubtedly did in the evolution of new species, 36-chromosomed forms will be produced. Such forms, from a theoretical point of view, ought to contain, at least 4 pairs of short chromosomes. This is actually the case in many of the 36-chromosomed species, particularly in *Dypsis madagascariensis*. In a number of other species with $2n = 36$ chromosomes, where all the 4 pairs of short chromosomes have not been made out, this may be due to the lack of an appreciable difference in length between the smallest medium-chromosomes and the short ones.

There is again ample evidence to show that such increase in number of the chromosomes by fragmentation has actually taken place in a number of cases. The critical studies made by Heilborn (1924) on different species of *Carex* have gone a long way to establish the importance of fragmentation. Belling (1925) has also explained the origin of the 16-chromosomed variety of *Secale cereale* from the usual 14-chromosomed types by fragmentation. Transverse septation has also played a part in the evolution of the different aneuploid series met with in *Tecoma* (Venkatasubban, 1945).

It is probable that the early establishment of a number of secondary base numbers from the primary one is one of the reasons for the success achieved by the members of certain families. For example, the genus *Crepis* belonging to the Compositæ has a number of base numbers like 3, 4, 5, etc., all derived from the primary base number of 4 chromosomes (Babcock and Cameron, 1934). Again, Fagerlind (1937) has recorded the base numbers 9, 10, 11, 12, 14 and 17 in the family Rubiaceæ. If the antiquity of a family and its numerical strength are the criteria of its advanced nature and success, then, the Palmæ also can, no doubt, be ranked as one of the most successful and advanced families, in which case, the early establishment

of two secondary base numbers acquires an added significance. This view is of course expressed only in a tentative manner at present as it is likely that these suggestions may have to be modified when further information is available regarding chromosome numbers and other cytological features occurring in the family.

Of the three base numbers suggested, 16 and 18 seem to have been highly successful, judging from the frequency of their occurrence, 16 in 12, out of the 14 investigated species of the tribe *Ceroxylinæ* and 18 in 18 out of the 20 species belonging to the tribe *Coryphinæ*. Incidentally it may be remembered that the tribe *Ceroxylinæ* is the largest of the 5 tribes and contains 85 genera. Next comes the *Coryphinæ* which has 22 genera while the other tribes *Borassinæ*, *Lepidocarynæ* and *Phytelephantinæ* contain, respectively, 6, 16 and 2 genera. If the chromosome number determined for a solitary member of the genus *Calamus* can be taken as representative of the entire tribe, then the *Lepidocarynæ* is likely to have adopted the secondary base number 14. *Borassinæ* appears to have taken to both 16 and 18 and the tribe *Phytelephantinæ*, which contains only a couple of genera, to 16 as the base number.

(*b*) *Speciation.*—The data on hand, although inadequate, also provide some clue regarding speciation. In the two species of *Thrinax* there are almost identical sets of chromosomes. This is also true of the different species of *Kentia* where the chromosomes agree both in their morphology and number. Differences in the species, in such cases, may perhaps be due to different dispositions of the genes or other structural changes. The 9 different species of *Phœnix*, which have been investigated and which possess the same chromosome number, probably, come under this category. Secondly, there are species which agree in their chromosome number but there is a size difference. This is true of the two species of *Areca*. The chromosomes of *Areca catechu* are appreciably bigger than those of *A. lutescens*. The same is the case with the two species of *Sabal*. Here also the chromosome complements of the two species disagree in their morphology and attention has been drawn to this already. Lastly, come those species which exhibit differences both in chromosome number and morphology. This is best seen in the three species of *Pritchardia*. The chromosome complements of *P. pacifica* and *P. filifera* are quite identical, but they differ considerably from the chromosomes of *P. grandis*. Here we have, therefore, an instance of species formation through a change in the size as well as the number of the chromosomes.

V. Summary

A study has been made of the root-tip chromosomes of 30 species of Palmæ spread over 23 genera.

From a study of the frequency of the chromosome numbers, for the investigated members of this family, it is suggested that 16 is the primary base number and two secondary base numbers, 14 and 18 were derived due to fusion and fragmentation of some of the chromosomes.

The question of speciation is also discussed for some of the members of the family.

VI. Acknowledgments

The author is greatly indebted to Dr. P. Maheshwari, of the Dacca University, for going through the manuscript and for his valuable suggestions and criticisms. Part of the work was done in the Botanical Laboratory of the Annamalai University and thanks are due to Prof. T. S. Raghavan for the facilities extended.

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Explanation of Text-Figures

All drawings were made at bench level with a camera lucida and a Zeiss Flourite objective ×100 in conjunction with Zeiss eye-piece K, 30 giving an approximate magnification of 5,200 diameters.

Text-Figs. 1–10 B.—Fig. 1. Somatic metaphase, polar view ($2n = 36$) of *Phœnix paludosa* Fig. 2 A. Somatic chromosomes ($2n = 36$) of *Raphis flabelliformis* seen in the polar view of metaphase. Fig. 2 B. Somatic metaphase chromosomes of the same species arranged according to their lengths. Fig. 3 A. Somatic metaphase chromosomes of *Thrinax excelsa* seen in polar view ($2n = 36$). Fig. 3 B. Idiogram of the same. Fig. 4 A. Somatic metaphase in *Thrinax argentea* ($2n = 36$). Fig. 4 B. Idiogram of the same species. Fig. 5. Somatic metaphase of *Licuala peltata* ($2n = 28$). Note the small size of the chromosomes compared to those found in *Thrinax* species. Also note the presence of 4 big chromosomes within the complement. Figs. 6, 7, 8 and 9 represent somatic metaphase plates of *Livistonia chinensis*, *Livistonia rotundifolia*, *Pritchardia pacifica* and *Pritchardia filifera* respectively all showing $2n = 36$ chromosomes. Figs. 10 A and 10 B. Somatic metaphase plate and idiogram of *Pritchardia grandis* ($2n = 16$). Note that the chromosomes of this species are different from the other two species both in size and number.

Text-Figs. 11–19 B (×5200).—Figs. 11 and 12. Somatic metaphase chromosomes of *Sabal adansoni* ($2n = 36$) and *S. umbracaulifera* ($2n = 36$) respectively. Note the presence of two long pairs of chromosomes AA′ and BB′ in the former and how they are different from the two long pairs of chromosomes A A′ and BB′ in the latter. Fig. 13. Somatic metaphase plate of *Latania rubra* ($2n = 32$). Fig. 14. Somatic complement in *Borassus flabellifer* ($2n = 36$). Note the big size of the chromosomes with sub-median constrictions. Figs. 15 A and 15 B. Somatic chromosomes of *Calamus* sp. ($2n = 28$) and idiogram of the same. Note the presence of two long pairs of chromosomes AA′ and BB′. Figs. 16 A and 16 B. Chromosome complement in the root cell ($2n = 32$) and idiogram of *Arenga saccharifera*. Figs. 17 A and 17 B. Somatic metaphase chromosomes and idiogram of the same ($2n = 26$) in *Dypsis madagascariensis*. Figs. 18 A and 18 B. Somatic chromosomes and idiogram ($2n = 36$) of *Oreodaxa regia*. Figs. 19 A and 19 B. Somatic complement ($2n = 32$) and idiogram of *Howea forsteriana*.

Text-Figs. 20 A–27 B. Somatic metaphase plates of various species of the tribe *Ceroxylinæ* and their idiograms drawn at an approximate magnification of ×5200 except Fig. 27 A which is ×3600. All the figures have been reduced to one half the size in reproduction.—Figs. 20 A and 20 B. *Heterospatha elata* ($2n = 32$). Note the 4 pairs of long chromosomes, two with median constrictions and the other two with sub-median constrictions. Figs. 21 A and 21 B. *Nephrosperma van houtteana* ($2n = 32$). Figs 22 A and 22 B. *Oncosperma filamentosa* ($2n = 32$). Figs. 23 A, 23 B, 24 A and 24 B. Somatic metaphase plates and idiograms of *Kentia MacArthuri* ($2n = 32$) and *Kentia sanderiana* ($2n = 32$) respectively. Note the presence of 3 pairs of short chromosomes S_1, S_2 and S_3 in the idiograms of both the species. Note also the presence of 4 long pairs of chromosomes L_1, L_3, L_5 and L_4 in both. Figs. 25 A and 25 B. *Dictyosperma album* ($2n = 32$). Figs. 26 A and 27 A. Somatic complements of *Areca lutescens* ($2n = 32$) and *A. catechu* ($2n = 32$) respectively. The idiograms of these two species are represented in Figs. 26 B and 27 B respectively. Note the presence of 2 pairs of very short chromosomes S_1 and S_2 in both these species.

Text-Figs. 28–30.—Figs. 28 and 29. Somatic metaphase plates as seen in polar view of *Cocos schizophylla* ($2n = 32$) and *Martenzia caryotæfolia* ($2n = 32$). ×5200. Fig. 30. Somatic metaphase plate of *Elæis guineensis* ($2n = 32$). ×*Ca* 2500.

SUB-GROUPS OF A AND AB HUMAN BLOOD*

With Particular Reference to Distribution of A_1 A_2 BO System of Blood in Indians

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Received April 17, 1945
(Communicated by Dr. V. R. Khanolkar, F.A.Sc.)

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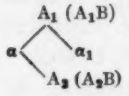
I. Review of Literature

Landsteiner[1a] in 1900 discovered three human blood groups, and Decastello and Sturli pursuing the study found in 1902 the fourth group. The four groups were subsequently designated as O, A, B, and AB. In 1910,

* This study forms a part of the thesis for Ph.D. Degree of the Bombay University. The work is at present in progress at the Tata Memorial Hospital under the guidance of Dr. V. R. Khanolkar and is aided by a grant from Mr. A. D. Shroff. The author is indebted to the authorities of Nowrosjee Wadia Maternity Hospital and to Major P. Warmold, I.M.S., for facilities in collecting blood.

von Dungern and Hirszfield[16b] first pointed out that when a group B serum was absorbed with certain group A cells until it lost the power of agglutinating the absorbing cells, the serum still retained the power of agglutinating a good many other cells of group A and group AB. On the basis of this observation, they suggested the existence of two sub-groups within each of the groups A and AB, based on differences in the agglutinogen A. These results were confirmed by subsequent investigators.[1, 7, 11]

This phenomenon is apparently due to the presence of two isoagglutinins in most group B and group O sera; α which agglutinates all group A and group AB erythrocytes and α_1 which agglutinates many but not all A and AB erythrocytes. The bloods agglutinated both by α and α_1 are designated as A_1 and A_1B: those agglutinated only by α are A_2 and A_2B.



Lattes[16c] proposed that the difference between the sub-groups is merely quantitative in character, caused by varying amounts of the same, agglutinogen in the two sub-groups and he and Cavazutti[16d] showed that both agglutinins α and α_1 which are present in group B serum can be removed completely by absorbing it with a large excess of A_2 cells. Landsteiner and Witt[11] however showed that the two agglutinins α and α_1 can be separated experimentally. Landsteiner and Levine[9] therefore proposed in 1926 that the difference between the sub-groups is a qualitative one. To clear up this difference of opinion, Friedenreich and Worsaæ[5] carried out a systematic quantitative examination of the absorption power of the corpuscles in one hundred group A individuals. The persons separated according to the absorption power into two groups, one of strong and another a much weaker absorption. It was observed that there was no transition between the two groups. The strong and the weak types were found on subsequent direct comparison to be identical with Landsteiner's A_1 and A_2. Weiner's[15] observation in 1941, that individuals of groups A_2 and A_2B can be immunised against A_1 bloods by repeated transfusions, or in case of women, by bearing a child whose blood contains the antigen A_1 may be assumed to support the same hypothesis.

Terminology of the sub-groups is not very happy because corresponding to the agglutinogens A_1 and A_2 in the cells, there are not α_1 and α_2 naturally occurring agglutinins in all group B or group O sera; nor has any investigator so far been successful in demonstrating the presence of α_2 in any human B or O group sera. Coca and Klein[1] found that seven out

of twenty-five B group sera contained little or no agglutinin α_1. It is for this reason that Taylor and his collaborators[14] suggest that the nature of the sub-groups may probably be most easily made clear by assuming two types of antigens A and A_1 and that sub-group A_1 contains both these antigens while A_2 has only A; corresponding anti-bodies α and α_1 are found in most group B sera.

A small percentage of groups A and AB individuals have in their sera irregular agglutinins α_1 and α_2 reacting with bloods of opposite sub-groups. Landsteiner and Witt[11] found α_1 in the sera of 5 out of 30 persons of group AB. Some of these reactions were detected only at low temperature. Wiener[15] found α_1 in 4 out of 13 A_2B cases and in none of 98 A_2 cases. Taylor and his collaborators[14] found α_1 in 74 out of 283 A_2B cases (26 %) and in 62 out of 3871 A_2 cases (1·6 %). Irregular agglutinin α_2 which can occur only in A_1 and A_1B bloods has the peculiarity of reacting even more strongly with group O bloods than with A_2 bloods. For this reason it is also designated as anti-O agglutinin. A method of preparing this agglutinin in experimental animals has been described by Wiener.[16a]

The importance of the sub-groups in connection with transfusion therapy is two-fold. Firstly, sub-groups A_1 and A_2 or A_1B and A_2B are not always compatible due to the presence of irregular agglutinins α_1 and α_2. The use of a donor of sub-group A_1 for a recipient of sub-group A_2 is more likely to give reactions than the reverse procedure because (as stated above) α_1 is commoner in A_2 bloods than α_2 is in A_1 bloods. The corresponding risk is greater when the donor belongs to sub-group A_1B and the recipient to sub-group A_2 B, as 26% of A_2B bloods contain the agglutinin α_1 in their sera. Even when α_1 is not present in the A_2 or A_2B group serum of the recipient at the time of first transfusion, A_1 or A_1B blood—if transfused—leads to the development of this agglutinin which may cause reactions during subsequent transfusions. Secondly,[17] A_1 is generally more sensitive to agglutination than A_2 ; and reactions of AB bloods with group B sera are weaker than those of bloods containing agglutinogen A alone. The relative sensitivity of A agglutinogen in the four sub-groups can accordingly be indicated as: $A_1 > A_1B > A_2 > A_2B$. It is for this reason that individuals of sub-groups A_2 and A_2B are sometimes mistaken for groups O and B respectively if weak group B typing serum is used. This mistake might not be detected by testing the serum which may contain the agglutinin α_1 which clumps the A_1 testing cells. Bloods from such mistaken universal donors who belong in reality to group A_2, are likely to produce strong reactions particularly in O or B group recipients. These are not purely theoretical considerations as reactions of varying intensity, sometimes even fatal have been reported by

Davidsohn.[2] In majority of these cases, the reactions could have been avoided by a careful matching of cells of the donor with the serum of the recipient.

The relative frequency of agglutinogens A_1 and A_2 in most white races is between 3 : 1 and 5: 1.[17] In 82 Negroes of group A examined 49 belonged to sub-group A_1 giving a relative frequency of 3: 2,[10] and suggesting a racial difference with regard to these sub-groups.

The genetic theory for the sub-groups is an extension of Bernstein's theory of the three allelomorphic genes A, B and R for the four classical groups. Thomsen, Friedenreich, and Worsaæ[16f] proposed the existence of four allelomorphic genes A_1, A_2, B and R with genes A_1, A_2 and B dominant over gene R and A_1 dominant over A_2.

Further subdivision of group A individuals has been reported by Friedenreich[4] in 1936. Certain individuals of group A reacted very feebly even with the most potent group B sera. He explained this phenomenon by postulating a third sort of A agglutinogen, designated by him as A_3 and giving rise to two additional sub-groups A_3 and A_3B. Among 5,000 individuals he found only 6 A_3 individuals. Cases of such unusually weakly reacting A antigen have been described by Wiener[17] and by Taylor.[13] Gammelgaard and Marcussen[6] have discovered a fourth sub-type of A antigen designated by them as A_4.

II. The Problem

Blood group investigations have been very few in India, as compared with some other countries of the world. Even among these few, most are lacking either in technical accuracies or statistical competance.

The unit for blood group classification is an endogamous unit, which has reached a stage of random equilibrium by inter-marriages through several generations. Most of the countries of the world may be considered as single endogamous units for purposes of classification. The fact that the Indian population consists of a very large number of endogamous units, spread over the length and breadth of the country, does not permit an easy classification applicable to the whole country and necessitates a sorting out of the data on the basis of known endogamous units.

The initial difficulties in blood group investigations are generally related to the obtaining of standard sera. Standard sera for ABO system are easy to obtain. Technical difficulties for preparing standard sera for the extended ABO system and for other systems of blood are many.

The present investigation was undertaken as a preliminary study:—

1. to clear technical difficulties attending various systems of blood;
2. to find whether a relationship exists between blood groups and some diseases; and
3. to determine the affinities or distinctions between different endogamous groups of population.

III. Material and Methods

To test a blood for sub-groups A_1 and A_2 or A_1B and A_2B—after determining that it belongs to group A or AB—the standard serum containing the antibody α_1 can be freed from α by mixing group B or group O serum containing both the agglutinins α and α_1 with a suitable quantity of group A_2 cells. These cells absorb the anti-body α and leave α_1 in the serum.

Initial difficulty of locating an A_2 individual was overcome by a titration experiment of 8 different A group cells all titrated with the same serum No. 3981 having a titre of 256. The cells were washed three times and resuspended in normal saline to give a 2·5% suspension in terms of blood sediment. The serum was diluted in 6 tubes serially from 1 : 16 to 1 : 512. The tests were set up in small tubes (2″ × ¼″ internal diameter), each tube receiving 0·1 c.c. of cell suspension and 0·1 c.c. of diluted serum. The racks were well shaken and allowed to stand at room temperature. The readings were taken after 2 hours and overnight. A sample protocol of an experiment with readings after 2 hours is given below:—

Table I

Sensitiveness of Group A Cells

Group A Cells Number	Serum Dilution (Group B No. 3981)						
	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Saline Control
3965	C	V	++	+	+	–	–
3966	C	V	++	++	+	–	–
3967	V	V	++	+	+	–	–
3968	V	V	++	+	±	–	–
3970	C	V	++	++	+	–	–
3974	V	++	++	+	+	–	–
3977	C	V	++	+	+	–	–
3979	++	+	+	–	–	–	–

C One large clump. + Only microscopically visible clumping.
V Several large particles. ± Doubtful clumping under microscope.
++ Small clumps readily seen by naked eye. – No clumping even microscopically.

There was no change in the results overnight, except in case of cells No. 3968, where the doubtful clumping showed a clear clumping. Blood No. 3979 was clearly weakly agglutinating and probably belonged to sub-group A_2. To confirm this result and to collect standard sera, 1 c.c. of these packed washed cells were mixed with 4 c.c. of two different B sera No. 3981 (titre 256) and No. 4032 (titre 128). The mixtures were allowed to stand for one hour at room temperature, centrifuged and the absorbed sera were separated. Both sera were tested by 8 different A group cells utilzed above. There was no clumping with cells No. 3979 and clumping in the rest as was expected.

In view of the difficulty of getting group B sera of high titre for preparing the standard sera, group O sera of high titre were used with advantage for testing the bloods of A group. So far 13 group B absorbed sera and 7 group O absorbed sera are prepared. In none of them agglutinin α_1 was absent. One volume of group B or group O serum of titre 128 or more was absorbed with $\frac{1}{4}$ volume of washed packed A_2 cells. The absorbed serum was tested by a suspension of absorbing cells. If the absorption was complete there would be no clumping. If there was clumping the serum was further absorbed by 1 : 16 volumes of the same cells and retested and the process was repeated till there was no clumping. In most cases there was complete absorption after 2 trials. The serum was not absorped with a large amount of A_2 cells in a single trial as it weakens the strength of agglutinin α_1 also.

Tests for sub-groups were carried out in small tubes set out in a metallic rack, each tube receiving one drop of normal saline, 1 drop of 2% cell suspension of unknown sub-group and 1 drop of standard serum. Each blood was tested by two different sera absorbed by two different cells of sub-group A_2. A_1 and A_2 controls were included in each experiment. The rack was well shaken and allowed to stand for one hour at room temperature. The rack was then shaken till the negative control went in clear suspension. The tests were read macroscopically and negatives under a microsocpe at low magnification (40 times). No reading was recorded as negative unless at least 3 fields under microscope showed a clear homogeneous spread of cells.

IV. Results

Our data consist of 1169 individuals typed for A_1A_2BO system with a record of information regarding caste, place of birth and sex. Table II summarises the relevant information.

TABLE II

Percentage Distribution of Groups of A_1A_2BO System

Caste or Race	No. of persons	Percentage of group						$\frac{A_2 + A_2B}{A_1 + A_1B}$
		O	A_1	A_2	B	A_1B	A_2B	
Marathas	289	37·4	27·3	3·1	22·5	8·0	1·7	·14
Mahars	153	37·9	20·3	4·6	31·4	5·2	0·6	·21
Other Hindus ..	445	33·9	25·8	4·3	27·9	5·6	2·5	·21
Musalmans ..	62	29·0	17·7	8·1	38·7	6·5	0·0	·33
Indian Christians * ..	110	38·2	23·6	4·6	30·9	2·7	0·0	·17
Europeans	100	45·0	31·0	9·0	13·0	1·0	1·0	·31
British (Ikin *et al.*) ..	3,459	43·5	34·8	9·9	8·6	2·6	0·6	·28

V. DISCUSSION

Out of 1169 individuals typed for A_1A_2BO system 3 Parsees, 4 Pathans, 1 Irani, 1 Jew, and 1 Chinese were excluded as they were small figures and could not be classified conveniently. In the remaining there were 880 Hindus, 62 Musalmans, 110 Indian Christians and 100 Europeans. In the group "Hindus" there were as many as 87 different castes each being an endogamous unit; some having further intermarital subdivisions. The numbers for castes Marathas and Mahars only were adequate for separate calculations, while the rest had been grouped together in the class " Other Hindus". "Musalmans"[3] was a heterogeneous group varying from individuals of a pure Musalman descent to converted individuals of pure Hindu descent with manners and customs of Hindus, and continuing as endogamous units, the intermediate range between the two extremes occupied with a variable mixture of the two. It was not possible to treat these endogamous units separately due to the small size of the data. The " Indian Christian" in this material is a relatively more homogeneous group consisting of converts mainly from low caste Hindus of Western Ghats.

In Table III are presented the estimated gene frequencies and the values of χ^2 for the castes Marathas and Mahars and also for Musalmans and Indian Christians by the method suggested by Stevens.[12]

A simple explanation for gene-frequencies may be suggested as under: according to the accepted genetic theory there are four allelomorphic genes R, A_1, A_2, and B and their frequencies are indicated by small letters r, p_1, p_2 and q respectively. So in a population of Marathas of 50,000 say, there will be 61,131 R genes, 19,309 A_1 genes, 2,496 A_2 genes and 17,064 B genes, and similarly for other classes. In any ideal population these

TABLE III

Estimated Gene-frequencies and Values of χ^2

Caste	Estimated gene-frequency				χ^2	Limits of P
	r	p_1	p_2	q		
Maratha	·61131	·19309	·02496	·17064	3·73	·2 > P > ·1
Mahar	·61570	·14699	·03610	·20121	1·22	·7 > P > ·5
Musalman	·53882	·14510	·07026	·24582	3·04	·3 > P > ·2
Indian Christian ..	·61791	·16833	·03576	·17800	4·86	·1 > P > ·05

frequencies r, p_1, p_2, and q should add to unity. Due to errors of random sampling, there will be a small difference. This difference is adjusted in q to give the total unity. The pheno-types O, A_1, A_2 B, A_1B and A_2B are recalculated on the basis of these adjusted frequencies. From the observed values and these expected values, χ^2 is calculated.

It has been possible to evolve a graphical method of representing the four genes p_1 p_2, q and r by a simple extension of the method adopted in representing the three genes p, q and r by Streng's triangle.[16,17] One more reference line can be drawn perpendicular to the reference line (p) at the point $p = 0$. The estimated gene-frequencies for a single race can then be represented by a line perpendicular from the point (p, q, r) to the new reference line and putting a mark for p_1 on this representative line. The same method can be extended to suit further subdivisions of the gene p.

VI. CONCLUSIONS

In order to check the agglutinin content of our standard absorbed sera, bloods of 100 Europeans were tested. The results are given in Table II. It will be observed that the distribution is very close to the one obtained by Ikin *et al.*[8]

Values of χ^2 in Table III are not significant for any class, showing that the distributions are in accordance with the accepted genetic theory, and assuming the genetic theory the data may be regarded as homogeneous.

In the last column of Table II is given the ratio $\left(\frac{A_2 + A_2B}{A_1 + A_1B}\right)$. The findings give an interesting anthropological hierarchy as follows

Marathas	·14
Indian Christians	·17
Other Hindus	·21
Mahars	·21
Musalmans	·33

The same ratio for American Indians is zero. While for Negroes (U.S.A.) it is ·37.[16] The figure for Musalmans is quite distinct from the other four classes while it is near the Negroes (U.S.A.), and seems to remind one of the historical evidence of a mixture of negrito blood in Musalmans in India. It has however to be admitted that the difference might also be due to the small size of the sample.

VII. Summary

1. The data consist of 1169 individuals, including 1069 Indians and 100 Europeans, typed for A_1A_2BO system and classified according to their caste, community or race. The ratio $\left(\frac{A_2 + A_2B}{A_1 + A_1B}\right)$ gives an interesting anthropological hierarchy.

2. A protocol of the experiment to locate an individual of group A_2 is given. The technique of preparing the absorbed sera and the method of testing the sub-groups are given in deteail.

3. The data are analysed statistically by estimating the gene-frequencies and calculating the values of χ^2. The values of χ^2 are in no case significant showing that the data are homogeneous.

4. A method of representing graphically the gene-frequencies p_1, p_2, q and r is suggested.

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841.48 — Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore

STUDIES ON COTTON JASSID (*EMPOASCA DEVASTANS* DISTANT) IN THE PUNJAB*

VII. Age of Leaf and Jassid Susceptibility

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Received December 14, 1944

(Communicated by Rao Bahadur V. Ramanatha Ayyar, F.A.SC.)

Introductory

Opinion regarding the preference, if any, shown by jassids (*Empoasca* sp.) for leaves of different ages for feeding and egg-laying appears to be divided. Smith and Poos (1931) have carried out extensive studies on six species of jassids and as a result of this work they have divided these species into two groups, *viz.*, those which feed on the mesophyll tissue of leaves and the others wherein feeding is confined to the phloem tissue. In the former case the "more mature foliage seems to be preferred to that of more succulent younger leaves" while in the latter case "fresh phloem tissue in succulent plants" is required for the well-being of the insect. A. W. S. May (private communication) found that in Queensland the cotton jassid (*E. maculata*) attacks the terminal leaves which are very young and succulent in preference to the older leaves. It must, however, be mentioned that so far as we are aware no results of any well planned experiments to find out the most preferred age of leaves have so far been reported. This point is of considerable importance as the so-called "jassid effect" (Mac Donald, Ruston and King, 1944) or the "symptom picture" (Wells, 1943) can be best judged from the zone of the plant which is liable to the worst attack.

The feeding habit of the cotton jassid (*E. devastans* Distant) in the Punjab appears to be entirely different from that of the six species under experiment by Smith and Poos. In this case there is neither "stippling" nor is the entire leaf ever wilted. The "jassid effect" in the present case is that there is a marked crinkling of leaves due apparently to the proliferation of mesophyll tissue. The leaf margins dry up and the crinkled area first becomes brick-red and ultimately dries up. It will thus be of great interest to find out the exact feeding habits of this species and work on this aspect of the problem has now been started.

* The previous articles of this series appeared in the *Indian Journal of Agricultural Science* and the *Indian Journal of Entomology*.

There is another aspect of this problem. In any samp'ing study where the leaf area is to be correlated with the number of jassids, it is clearly of importance to take those leaves on which the oviposition is highest and in any comparative study, leaves from the same plant zone should be taken. Thus the most preferred age of leaves for oviposition should be determined for different species before any such work is undertaken.

Work on this problem has been in progress at the Cotton Research Laboratory, Lyallpur, and the present preliminary report is published for information of other workers. Detailed data covering a large number of years and varieties of cotton will be published later.

Material and Method

Two varieties of American cotton (*G. hirsutum*) one highly resistant (199 F.) and the other highly susceptible (289 F./K·25) to jassid (*Empoasca devastans* Distant) were sown on a separate plot at the normal time of sowing (17–6–1943). Five normal plants of each variety were selected in the very early stages of growth and the leaves on the main stem were tagged on the date of separation from the growing point. Thus the age of all the leaves on the main stem was known.

As the jassid lays its eggs inside the leaf vein, the only method of studying the oviposition would be the dissection of the leaf vein. This would necessitate the plucking of the leaf from the plant. This method would thus be obviously unsuitable in the present studies. The oviposition was, therefore, studied indirectly by counting the number of nymphs on all the leaves on alternate days. The nymphs were killed after counting and removed from the leaf with a camel hair brush. Thus at the end of the experiment the number of nymphs found on all the leaves of different ages was calculated. For obvious reasons the experiment was confined to the primary leaves only, but there is no reason to suppose that the secondary or the tertiary leaves would differ in their behaviour from the primary leaves. In the studies now under way, however, all leaves are being studied.

It has been elsewhere proved (Verma and Afzal, 1940) that the eggs of *E. devastans* when laid in the leaf veins of even immune varieties like *G. arboreum* have no difficulty in hatching. Thus the number of nymphs found on the leaves of different ages would indirectly show the preference of the insect for egg-laying.

Data and Results

The tabulated figures of the number of nymphs found on leaves of different ages in both the varieties are given in Table I and are graphically shown in Fig. 1

TABLE I

Calculated and observed values of the nymphal population per leaf per day

Variety	Age of leaf	Nymphal population		Age of leaf	Nymphal population		Age of leaf	Nymphal population	
		Observed	Calculated		Observed	Calculated		Observed	Calculated
	1	0·000		23	0·453	0·613	45	0·697	0·743
	2	0·000		24	0·495	0·646	46	0·523	0·720
	3	0·000		25	0·514	0·676	47	0·543	0·694
	4	0·000		26	0·755	0·703	48	0·441	0·666
	5	0·000		27	0·742	0·728	49	0·410	0·635
	6	0·000		28	0·731	0·751	50	0·407	0·601
	7	0·000		29	0·677	0·771	51	0·434	0·565
	8	0·000		30	0·614	0·788	52	0·319	0·527
	9	0·000		31	0·874	0·803	53	0·283	0·486
	10	0·000		32	0·875	0·815	54	0·256	0·442
	11	0·011		33	0·724	0·825	55	0·184	0·396
199 F.	12	0·033	0·086	34	0·857	0·832	56	0·229	0·347
	13	0·131	0·147	35	0·892	0·837	57	0·219	0·296
	14	0·211	0·205	36	0·726	0·839	58	0·226	0·242
	15	0·245	0·260	37	0·753	0·839	59	0·095	0·185
	16	0·242	0·313	38	1·132	0·836	60	0·143	0·126
	17	0·531	0·364	39	1·247	0·830	61	0·250	0·065
	18	0·588	0·412	40	1·197	0·822	62	0·250	0·001
	19	0·476	0·457	41	1·039	0·811	63	0·273	−0·066
	20	0·476	0·500	42	0·986	0·798	64	0·250	
	21	0·583	0·541	43	1·133	0·783	65	0·000	
	22	0·491	0·578	44	1·041	0·764			
	1	0·000		23	1·142	1·273	45	1·214	1·494
	2	0·000		24	1·398	1·340	46	1·314	1·442
	3	0·000		25	1·571	1·401	47	1·270	1·385
	4	0·000		26	1·443	1·457	48	1·161	1·332
	5	0·000		27	1·436	1·507	49	0·985	1·254
	6	0·000		28	1·855	1·552	50	1·000	1·181
	7	0·000		29	1·759	1·592	51	1·000	1·102
	8	0·000		30	1·809	1·626	52	0·792	1·018
	9	0·000		31	1·897	1·654	53	0·524	0·928
	10	0·010		32	1·654	1·678	54	0·385	0·833
289 F/K. 25	11	0·566	0·056	33	1·667	1·696	55	0·515	0·733
	12	0·105	0·187	34	1·584	1·709	56	0·600	0·628
	13	0·200	0·313	35	1·646	1·716	57	0·583	0·517
	14	0·300	0·433	36	1·946	1·718	58	0·435	0·400
	15	0·396	0·548	37	2·211	1·715	59	0·429	0·278
	16	0·523	0·657	38	2·102	1·706	60	0·429	0·151
	17	0·613	0·761	39	1·988	1·692	61	0·250	0·019
	18	0·750	0·860	40	1·744	1·672	62	0·286	−0·119
	19	0·905	0·954	41	1·735	1·648	63	0·000	
	20	0·957	1·042	42	1·649	1·617	64	0·000	
	21	0·991	1·124	43	1·568	1·581	65	0·000	
	22	1·024	1·202	44	1·487	1·540			

This table shows that the nymphal population increased with the maturity of leaves in both the varieties, the maximum number of nymphs being observed on leaves of ages between 35 to 45 days in the case of 199 F. and between 30 to 40 days in 289 F./K. 25. Another fact that is brought out

by the data is that the peak in the case of a susceptible variety was very high as compared to the resistant variety.

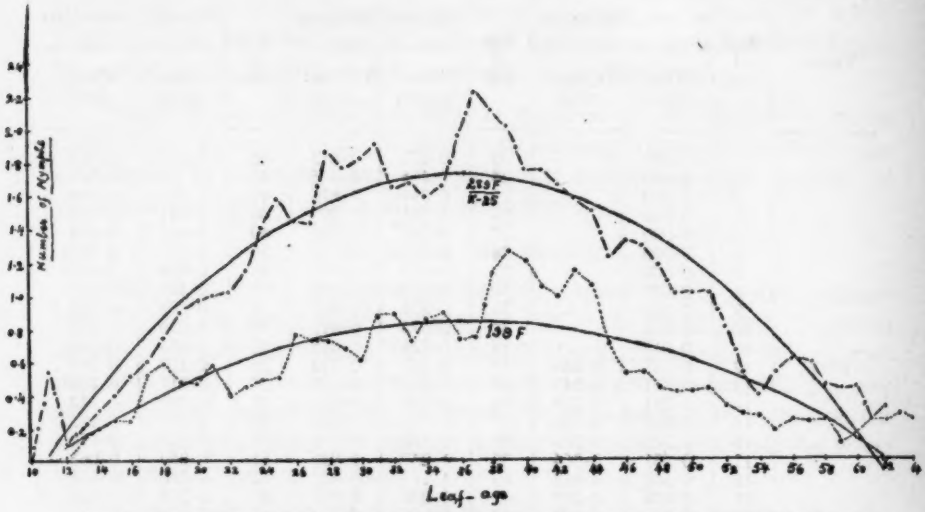

FIG. 1. Figure showing the relation between the number of nymphs and the leaf age

Polynomial curves of the third degree were fitted to the data of both the varieties, with nymphal population as the dependent and age as the independent variate. The sum of squares attributed by the linear, quadratic and cubic regressions along with the residuals are given in Table II.

TABLE II

Variance due to	D.F.	199 F.		289 F./K. 25	
		S.S.	M.S.	S.S.	M.S.
Linear Regression ..	1	0·103, 994	0·103, 994	0·138, 509	0·138, 509
Quadratic Regression ..	1	3·412, 654	3·412, 654†	15·256, 977	15·256, 977†
Cubic Regresion ..	1	0·076, 496	0·076, 496	0·238, 211	0·238, 211*
Residuals	48	1·472, 807	0·030, 683	2·077, 108	0.043, 273
Total ..	51	5·065, 951	..	17.710, 805	..

* Significant upto 5% level. † Significant upto 1% level.

It will be seen from Table II that the variation in the nymphal population clearly followed a quadratic trend in both the varieties, although in the susceptible variety even the cubic regression was significant. This trend

of variation in the nymphal census points to the fact that the insect prefers mature leaves for oviposition while the young and the very old leaves are left alone.

The second degree parabolas (Fig. 1) fitted to the data are given below:—

$$199\ \text{F}:\quad Y = -0{\cdot}839{,}609 + 0{\cdot}092{,}420\,x - 0{\cdot}001{,}272\,x^2$$

$$289\ \text{F/K}{\cdot}25:\quad Y = -1{\cdot}741{,}029 + 0{\cdot}192{,}936\,x - 0{\cdot}002{,}690\,x^2$$

where Y = Nymphal population.

x = Age of leaf in days.

The difference in the span of the two curves can be observed by the ratio of the latera recta, which is 2·120, showing a highly peaked nature of the curve in 289 F./K. 25 and a flat peaked nature in the case of 199 F.

The rate of increase of the nymphal population can be determined by $\frac{dy}{dx}$ and the rate of this increase by $\frac{d^2y}{dx^2}$. These values are given below:—

	$\frac{dy}{dx}$	$\frac{d^2y}{dx^2}$
199 F.	$+ 0{\cdot}092{,}420 - 0{\cdot}002{,}544x$	$- 0{\cdot}002{,}544$
289 F./K.25.	$+ 0{\cdot}192{,}936 - 0{\cdot}005{,}380x$	$- 0{\cdot}005{,}380$

The values of $\frac{dy}{dx}$ and $\frac{d^2y}{dx^2}$ also point to a peaked nature of the curve in the case of 289 F/K·25 and flat one in 199 F. The co-ordinates of the vertices of the curves are 0·839 and 36·33 for 199 F. and 1·719 and 35·86 for 289 F./K. 25. These values show that the maximum nymphal population was observed on the leaves of almost the same age, *i.e.*, 36 days in both the varieties and that the maximum population in 289 F./K. 25 was more than double of that in 199 F.

The two findings brought out by the mathematical working of the data have now to be explained. The first finding is the attraction of the pest to a leaf of a particular age and the second is the difference in the kurtosis of the two curves. Due to the lack of direct experimental evidence the following probable explanations suggest themselves.

As the leaf separates from the terminal bud, its area is small but the hair density per unit area is great and consequently the leaf perhaps offers greater resistance to oviposition. When it grows and expands, the number of hairs probably remaining the same, the hair density falls and so the pest finds it easier to lay its eggs in the leaf veins. That the hair density decreases with the age of the leaf is confirmed by the finding at the Cotton Research

Station, Barberton (MacDonald, Ruston and King, 1944) where it was found that the leaves nearer the terminal bud are smaller and carry "denser and shorter hairs, with a higher average number of hairs per bunch, than those of the older and larger leaves lower down the stem". When the age advances, the oviposition increases until after a certain stage the leaf vein becomes very woody with the result that the old leaves are no more an attraction to the pest. In the very old age the hair density decreases as the hairs are d ciduous and fall off but due to the hardness of the veins the pest does not like to lay its eggs in them. Apart from the low hair density at the most attractive age of the leaf, the nature of the cell sap may be an additional attraction.

A more peaked nature in the susceptible variety as compared to the resistant one may be explained on the basis that the leaves of 199 F. are much more hairy than those of 289 F./K. 25. The hair density in 289 F./K. 25 is therefore, never of the same magnitude, as in the case of 199 F. Thus the leaves of 289 F./K. 25 offer comparatively less resistance for oviposition at all ages than the leaves of 199 F. It, however, appears that the rate of hardening of the leaf vein is similar in both cases, therefore, the fall in oviposition in 289 F./K. 25 is also more rapid than in the case of 199 F. This point is now being investigated, and the present finding is, therefore, only tentative.

Summary

The present is a preliminary report about the age of cotton leaves preferred for oviposition by *Empoasca devastans*. It has been shown that the insect prefers leaves of about 35 to 45 days age. The younger and the older leaves are usually left alone.

This finding is very important in breeding varieties for jassid resistance as the worker must look for the "jassid effect" in the middle zone of the plant rather than at the top or the bottom.

Acknowledgment

This work was carried out as a part of the Jassid Research Scheme financed by the Indian Central Cotton Committee.

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A CONTRIBUTION TO THE EMBRYOLOGY OF *ALANGIUM LAMARCKII* THW. WITH A DISCUSSION OF THE SYSTEMATIC POSITION OF THE FAMILY ALANGIACEÆ

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Received August 3, 1945

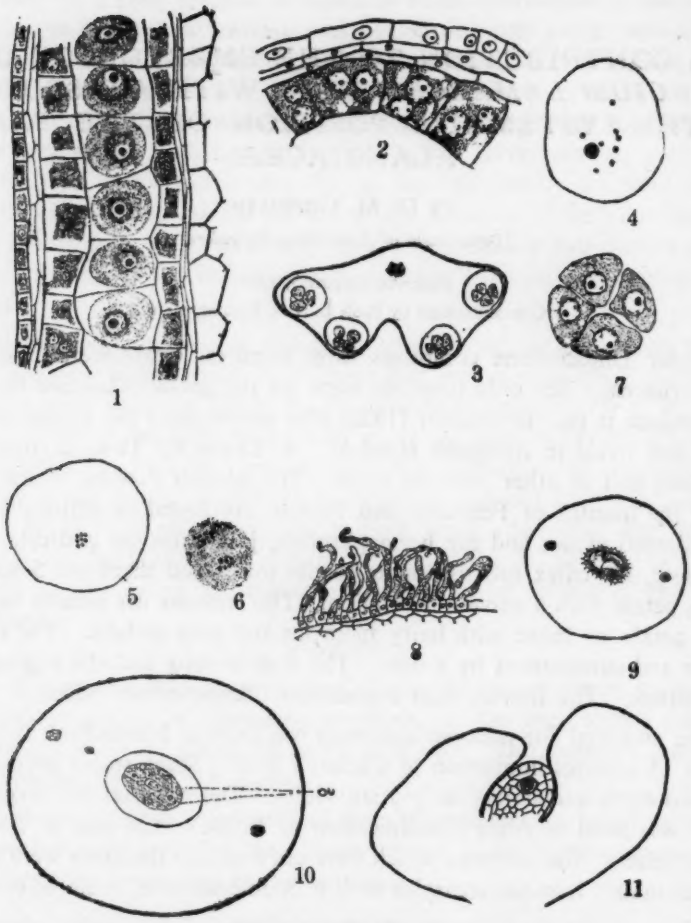
(Communicated by Prof. L. S. S. Kumar, F.A.SC.)

The order Umbellifloræ comprises three families, Umbelliferæ, Araliaceæ and Cornaceæ. The only previous work on the genus *Alangium* belonging to Cornaceæ is that of Schnarf (1922) who investigated the structure of the flower and ovule in *Alangium Handelli*. *A. Lamarckii* Thw. is common at Bangalore and in other parts of India. The whitish flowers, which appear during the months of February and March, are found in axillary fascicles or condensed cymes and are hermaphrodite, joined on the pedicel. Bracts are absent, the calyx tube is adnate to the ovary and there are 5–10 linear oblong petals with a valvate æstivation. The stamens are usually as many as the petals or more with hairy filaments and long anthers. The ovary is inferior and surmounted by a disc. The style is long and the stigma large and capitate. The flowers emit a peculiarly characteristic odour.

The material for microsporogenesis was fixed in Navaschin's fluid after a 10 to 15 minutes immersion in Carnoy's fluid. Some smear preparations were also made and stained in gentian violet. The material for megasporogenesis was fixed in Allen's modification of Bouin's fluid and in formalin-acetic-alcohol. The sections which were cut 6 to 18μ thickness were stained in Heidenhain's iron-hæmatoxylin with a counterstain of eosin in clove oil.

There are 20–30 stamens. A cross-section of the anther shows the usual tetra-sporangiate condition (Fig. 3). The parietal tissue consists of an epidermis, the endothecium, a single middle layer and the tapetum (Fig. 2). The tapetal cells are conspicuous owing to their prominent nuclei and soon become binucleate conforming to type II of Cooper (1933). Degeneration of the tapetum takes place as soon as the microspores begin to separate from each other.

A single row of sporogenous cells is differentiated in each lobe (Fig. 1). These are characterized by having dense cytoplasm and conspicuous nuclei.



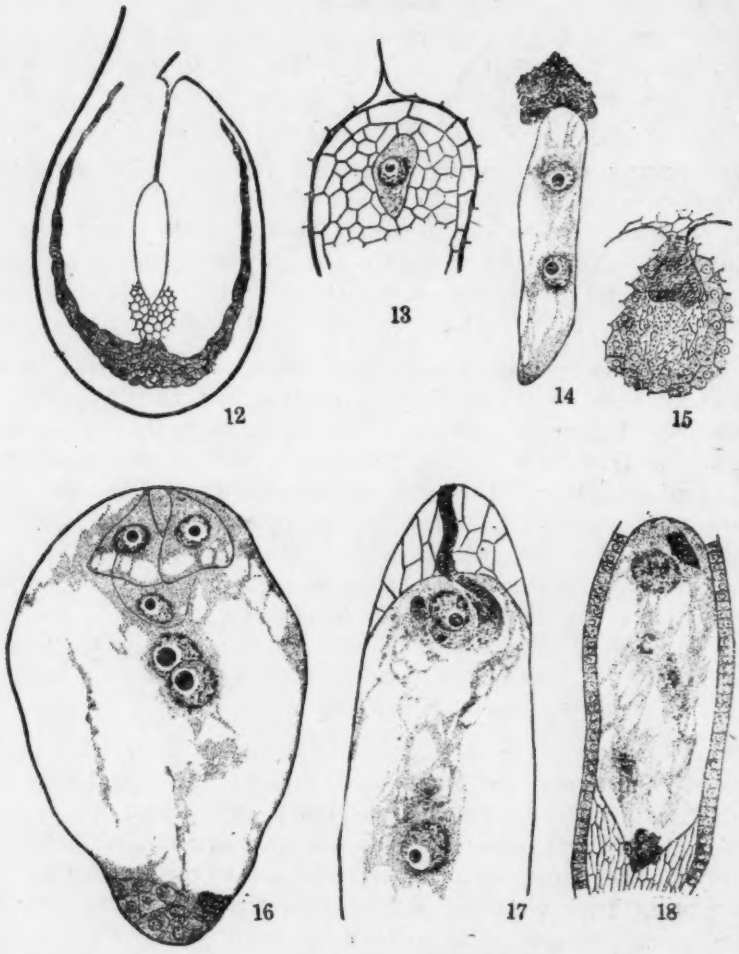
FIGS. 1–11.—Fig. 1. Longitudinal section of the anther showing the layer of sporogenous cells in one of the lobes. ×704. Fig. 2. T.S. part of anther showing the epidermis, endothecium, middle layer and binucleate tapetum. ×1900. Fig. 3. Diagram of cross-section of anther. ×400. Fig. 4. Same in diakinesis. ×1900. Figs. 5 and 6. Microspore mother-cell in reduction division showing eight chromosomes. Fig. 7. Tetrad of microspores. ×900. Fig. 8. A portion of the ovary wall enlarged to show the development of hairs. ×480. Fig. 9. Cross-section through the ovule to show the distribution of the vascular strands. ×480. Fig. 10. Cross-section of the ovary showing the distribution of vascular strands in the ovary and the ovule. OV, ovule; ES, embryo-sac. Fig. 11. Ovule showing sporogenous cell and single integument. ×704.

They undergo the usual reduction divisions to form tetrads of microspores. Polar views of the meiotic metaphase plates show the haploid number of chromosomes to be eight (Figs. 4, 5, 6). This has also been confirmed by counts in root tips where the diploid number was determined to be sixteen. The pollen at the shedding stage shows only one nucleus.

The ovary is inferior and unilocular (Fig. 10). In *A. Handelli*, Schnarf (1922) reports a bilocular ovary with one ovule in each locule, although only one of the two develops to maturity. The wall of the ovary is densely tomentose and this renders sectioning very difficult. The hairs originate from the epidermis and are uninucleate (Fig. 8). There is a single pendulous anatropous ovule as is characteristic of the Umbelliferæ.

There is a single massive integument which originates from the base of the nucellus but grows enormously (Fig. 11) so that the micropyle is long and narrow. The inner epidermis of the integument develops into an epithelial layer (Fig. 18) which has also been reported in *A. Handelli* (Schnarf, 1922), *Benthamia* (Horne, 1914), *Cornus suecica* (Morse, 1909) and *Aralia racemosa* (Ducamp, 1902). The nucellar cells disorganise on all sides of the embryo-sac except at the micropyle and chalaza (Figs. 17 and 18). At the chalazal end there is a persistent hypostase tissue composed of 4–5 layers of richly cytoplasmic cells. As in *A. Handelli* (Schnarf, 1922) there is a well-developed conducting strand consisting of elongated cells which are connected with the hypostase and continue further upwards upto the micropylar region of the integument (Figs. 9 and 12).

There is a single hypodermal archesporial cell which divides into a primary parietal cell and a megaspore mother cell (Figs. 11, 13). The latter undergoes the usual reduction divisions and forms a linear tetrad of megaspores of which the chalazal alone functions and the upper three degenerate (Fig. 14). The development of the embryo-sac is of the monosporic eight-nucleate type. The egg is fairly large in size and the polar nuclei meet very early but remain unfused till the time of fertilization. However this is not a constant feature and certain exceptional cases are noted where the polars fuse early. The antipodals are uninucleate in most cases and degenerate just prior to fertilization, but in some cases (Fig. 16) they show further development leading to the formation of antipodal embryos (Gopinath, 1943). It is a case of haploid apogamy. The entry of the pollen tube is of the usual porogamous type and in this process the small group of nucellar cells occurring on the top of the embryo-sac is also partially crushed and disorganised (Fig. 17). The endosperm is free nuclear in the initial stages (Fig. 18) but later becomes cellular, storing in its cells large quantities of



FIGS. 12–18.—Fig. 12. Longitudinal section through the ovule showing the course of the vascular supply in the integument. ×480. Fig. 13. Nucellus showing megaspore mother-cell. ×704. Fig. 14. Two-nucleate embryo-sac with remains of the upper three megaspores. ×1520. Fig. 15. Embryo with short suspensor embedded in a mass of endosperm cell. ×480. Fig. 16. Mature embryo-sac. Note the increased number of cells at the antipodal end. ×1140. Fig. 17. Fertilisation. ×475. Fig. 18. First division of the fertilized egg and free nuclear divisions of the endosperm. Note also the integumentary tapetum. ×440.

reserve food materials. The embryo has a short suspensor consisting of four to five cells (Fig. 15).

Discussion

The systematic position of the genus *Alangium* is of much interest. Bentham and Hooker (1879) place it under the Cornaceæ along with *Cornus, Acuba, Benthamia* and others. Engler and Prantl (1931) on the other hand have allocated a separate family Alangiaceæ with one genus *Alangium* and included it under the Myrtifloræ. Hutchinson (1926) while recognising the Alangiaceæ as an independent family, places it under the order Umbelliflorae. The latter view is also supported by Schnarf (1922).

The first point to be considered in this connection is the presence of a single integument which is common to both the Cornaceæ and *Alangium* (Brown and Horne, 1914). Wangerin (1910) however incorrectly ascribed two integuments to the ovule of *Alangium* (on the basis of Baillon's work) and removed it from the Cornaceæ to the Myrtifloræ, placing it between the Rhizophoraceæ and Combretaceæ. In the present study the author had an opportunity of making comparative studies of *Alangium* with some members of the Umbelliflorae including the Araliaceæ (Gopinath, 1944). It strongly confirms Schnarf's view that the Alangiaceæ should be treated as a separate family but included under the order Umbelliflorae. The Umbelliferæ, Cornaceæ and Araliaceæ have an inferior ovary, with a single pendulous anatropous ovule provided with a single integument. A similar condition occurs in *Alangium*. Again in many members of the Umbelliferæ also, including *Sium* (Coulter and Chamberlain, 1903) the nucellar cells are ephemeral and the integument forms an epithelial layer lining the embryo-sac. Some Araliaceæ investigated by the writer are also similar.

The chalazal strand observed in *A. Lamarckii* is also known in other families belonging to the Myrtifloræ, *viz.*, Lythraceæ (Joshi and Venkateswarlu, 1935–36), Thymeleaceæ (Kausik, 1940), Sonneratiaceæ (Venkateswarlu, 1937), but it seems to be related with the nutrition of the embryo-sac and might not have much systematic value and in any case the inferior ovary of *Alangium* with a single pendulous ovule and only one integument definitely speak against its relationship with the Myrtifloræ. Coming now to the position of the genus *Alangium* in the Umbelliflorae it has already been pointed out that Bentham and Hooker include it under the Cornaceæ without erecting a separate family for the purpose. The embryological evidence does not support such a view however. Thus, the multiple archesporium observed in *Acuba japonica* (Horne, 1941), *Benthamia capitata* (Jönsson, 1881), and *Cornus mas, C. suesica, C. florida* (Hakansson, 1923) is conspicuously absent in both the species of *Alangium* so far investigated. Further, in *A. Lamarckii* the antipodals sometimes persist till the time of fertilization

and occasionally divide further to form antipodal embryos, while in the Cornaceæ an early degeneration of the antipodals is universal. In the various members of the Cornaceæ so far investigated the heterotypic division in the megaspore mother cell is not accompanied by wall formation, with the result that four free nuclei are present of which one is functional (Schnarf, 1931; Morse, 1907). In *Alangium* on the other hand, a clear linear row of megaspores is formed. Again the type of endosperm formation, which is nuclear in *Alangium* and cellular in Cornaceæ, emphasizes the difference between the two. All these facts together with the upward extension of the vascular tissue into the integument which is found in *A. Lamarckii* and *A. Handelli*, but not known in any of the Cornaceæ clearly confirm Schnarf's opinion that the Alangiaceæ should be placed as an independent family under the Umbelliflorae.

Summary

The embryology of *Alangium Lamarckii* Thw. has been studied. A cross-section of the anther shows an epidermis, the endothecium, a single middle layer and the tapetum. The haploid number of chromosomes is eight. There is a single anatropous ovule with one integument. The development of the embryo-sac conforms to the Normal type and an epithelial layer is formed from the inner epidermis of the integument. Fertilization is porogamous and the endosperm nuclear. It is concluded that the genus *Alangium* should be placed in a separate family, the *Alangiaceæ*, under the order Umbelliflorae.

Acknowledgments

In conclusion, the author wishes to acknowledge with thanks the facilities and encouragement given by Dr. L. S. Dorasami, Economic Botanist, Department of Agriculture, Bangalore. He is also grateful to Dr. P. Maheshwari of the Dacca University for going through the manuscript and giving valuable suggestions and to Dr. M. J. Thirumalachar for kind help at various stages of the work.

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THE GENUS *QUERCUS* IN THE KAREWA DEPOSITS OF KASHMIR, WITH REMARKS ON THE OAK FORESTS OF THE KASHMIR VALLEY DURING THE PLEISTOCENE

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Received July 5, 1945

(Communicated by Professor Birbal Sahni, F.A.Sc., F.R.S.)

(With Four Plates)

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Introduction

The Lower Karewa Series of Kashmir, which are probably the best preserved Pleistocene deposits of India, have yielded, in recent years, from more than a dozen localities a rich fossil flora comprising mainly of Dicotyledonous families; however, some Monocotyledons and other plant groups, namely, Coniferales, Filicales and fresh-water algæ are also represented (Puri, 1939). The microscopic remains have been studied in some detail and about a hundred species of diatoms have been identified by Conger (see de Terra and Paterson, 1939, pp. 120–22) and fifteen species are described by Iyengar and Subrahmanyan (1943) from the Karewa shales; in addition to these, Wodehouse (1935) has described pollen grains of a dozen

species from a few pieces of clay sent to him by Dr. H. de Terra, the leader of the two Karakoram Expeditions to India in 1932 and 1935. A large number of specimens of an alga are identified with *Botrydium*; a detailed account of this interesting form is in preparation as a joint paper with Mr. Rama Nagina Singh of the Benares Hindu University.

Dr. de Terra presented a major part of his collections of the first Expedition, made at Liddarmarg (lat. 33° 48′; long. 74° 39′; alt. 10,600 ft.), Gogajipathri (lat. 33° 51′;long. 74° 41′; alt. 8,800 ft.) and Dangarpur (lat. 34° 8′; long. 74° 20′; alt. 6,500 ft.) to the Lucknow University for investigation under Professor B. Sahni, F.R.S. Prof. Sahni had originally entrusted the material to the late Dr. S. K. Mukerji, Reader in Botany at the University, and the latter before his death in 1934 was able to identify thirteen genera of fossil leaves, which were announced by de Terra (see Wodehouse and de Terra, 1935, p. 1) and later by Prof. Sahni (1936, p. 12) in an important paper giving a short account of the Karewas.

Although the macroscopic plant remains were known to occur in the Karewa deposits some 85 years back (Godwin Austen, 1859), no attention was paid to study this interesting flora till 1910 when the late Mr. C. S. Middlemiss (1911, p. 122) with the help of I. H. Burkill reported the occurrence of five different genera including *Quercus* from his collection from Liddarmarg.

All this material, which was unsatisfactorily examined before (see Puri, Paper I, unpublished in the Thesis) was later acquired by Prof. Sahni for investigation by the author, who later on supplemented it with his own collections from Laredura, Dangarpur and Botapathri. Further material was collected by Prof. Sahni in 1934 and 1936 from Dangarpur, Botapathri and several other localities; Dr. R. R. Stewart also made another collection from Laredura in 1936; thus all the material now comprising several thousand specimens which have so far been collected from the Karewas, have been made available to the author for study.

The present paper is devoted to the description of fossil leaves of *Quercus*, which on account of its best representation in the Karewa flora, both qualitatively and quantitatively, deserves a separate paper to itself.

I am highly indebted to Professor B. Sahni, F.R.S., for his guidance and helpful criticism during the preparation of this paper. Apart from the scientific guidance I have freely received other help from him without which this paper would never have been completed, for all that I take this opportunity of expressing my deepest debt of gratitude to him.

It is a pleasure to thank Dr. R. R. Stewart of the Gordon College, Rawalpindi, to whom I owe a great deal of my knowledge of the forest flora of the N. W. Himalayas and many a tips in identification of fossil leaves of *Quercus*. I am also grateful to Dr. N. L. Bor, formerly the Forest Botanist at the Forest Research Institute, Dehra Dun, and his assistant Mr. M. B. Raizada for help and facilities during my work at the Herbarium.

For loan of Middlemiss's collection and some photographs of *Q. glauca* and *Q. incana* from this material I am thankful to the Director, Geological Survey of India.

I must thank the Vice-Chancellor of the Panjab University and Principal Jodh Singh of the Khalsa College, Amritsar, for research grants from the University and the College and the authorities of the Lucknow University for award of a Research Fellowship. I am glad to acknowledge the help my wife has given me in correcting the proofs.

Plant-bearing Outcrops

Locality L.—Laredura, near Dangarpur, seven miles from Baramulla towards Gulmarg, height about 6,000 ft. above sea-level.

Locality 1 *D.*—One furlong east of Dangarpur village, at a steep cliff, 6,300 ft. above sea-level.

Locality 2 *G.*—Gogajipathri, one and half furlong towards the north of the upper houses at the village of that name, at 8,800 ft. altitude.

Locality 3 *L.*—Liddarmarg, in the stream bed 500 ft. west of the shepherd's huts, the beds dip 6 degrees north-east and are at 10,600 ft.

Locality K 14/948.—Liddarmarg, in two stream beds near the present Gujar (herdsmen) encampment, the dip of the beds is of the usual slightly inclined character, the altitude is 10,600 ft. above sea-level.

Locality B.—Botapathri, about 5 miles South of Dangarpur; the beds are exposed in a stream bed at an altitude of 9,500 ft. on south-west of the main huts of the village (Botapathri).

Systematic List of the Species

Quercus semecarpifolia Smith.
Quercus dialatata Lindl.
Quercus Ilex L.
Quercus incana Roxb.
Quercus glauca Thunb.
Quercus sp.

DISTRIBUTION OF THE SPECIES IN SPACE IN THE KAREWAS

Species	Laredura alt. 6.000 ft.	Dangarpur alt. 6,300 ft.	Gagajipathri alt. 8,800 ft.	Liddarmarg alt. 10,600 ft.	Botapathri alt. 9,500 ft.
Q. semecarpifolia ..	×	×	×		
Quercus dilatata ..	×	×	×		
Quercus Ilex	×	×	×		
Quercus incana				×	×
Quercus glauca				×	×
Quercus sp.				×	

DESCRIPTION

Family Fagaceæ

The family is represented in the Karewa flora by leaf impressions, which are identified with five well determined modern species of *Quercus*; in addition to leaves, there is also present one specimen of an impression from the outer flattened surface of a cupule, which also belongs to a species of *Quercus*. The Fagaceæ is one of the most important fossil families represented in the Karewa Formations. Oak leaves constitute nearly half of the entire fossil plant material from the Karewas, and they are found in great abundance at five different localities, which are not only distantly situated, but also lie at different altitudes.

It may be interesting to point out that fossil leaves of the three species—*Quercus semecarpifolia* Smith, *Quercus Ilex* L., and *Quercus dilatata* Lindl.—are found in hundreds in the beds that are exposed at Laredura (6,000 ft. to 6,500 ft. altitude), Dangarpur (6,300 ft. altitude), and Gogajipathri (8,800 ft. altitude), while the remaining two species, *viz.*, *Quercus incana* Roxb. and *Quercus glauca* Thunb. have not yet been discovered at these localities; however, they form a major part of the material which I have examined from Botapathri (9,500 ft. altitude) and Liddarmarg (10,600 ft. altitude); the first three species are not found at these two localities.

It may also be pointed out here that the five species, which during the Pleistocene times were probably growing in pure forests in this part of Kashmir, do not form a part of the present-day flora of the valley or the neighbouring hills of the Pir Panjal, but they have taken to the more favourable outer ranges, which receive heavy rains from the monsoon. This fact not only points out a contrast between the climatic conditions, that prevailed in the valley then and now but also throws considerable light on the past and present distribution of the Kashmir forests.

1. *Leaves*

Genus Quercus

Quantitatively, as well as qualitatively, *Quercus* is the best represented genus in the Karewa flora. Every other leaf in the entire material is an oak. In all the localities where it is found, it dominates over all other genera. Its representation in the Laredura beds is about 55% ; 60 to 70% of the specimens from Dangarpur and Gogajipathri belong to it, and it has the amazing percentage of 75 in the collections from the Liddarmarg clays. The only other genus which can at all compete with it in numerical strength is *Trapa*. The former is represented by leaves and the latter by fruits.

The specific determination of fossil leaves of *Quercus* is a difficult task because the living species exhibit an extreme range of variation in shape, size, nature of margins, base and apex within a species. Sometimes the variations are so bewildering that it becomes difficult to recognise normal variations within a particular species. Fortunately a large amount of well preserved fossil material was available and an intensive study of modern leaves belonging to numerous species of the genus both in the Herbarium and in the field has facilitated the identification of the fossil forms.

The fossil leaves of *Quercus semecarpifolia* Smith. and *Quercus Ilex* L. are usually not well preserved as regards details of the tertiary and finer reticulations; this may be partly due to the fact that the living leaves of these species have an indistinct and immersed venation, which is not well marked on either surface of the lamina. In the remaining three species, though the leaves are also quite thick, the venation is well marked on the lower surface and a few well preserved fossil leaves show up the venation quite clearly. The fossil leaves of *Quercus dilatata* Lindl. are usually the best preserved as regards venation and quite a few complete fossil leaves may be favourably compared to living leaves in this respect.

KEY TO THE SPECIES

I. Leaves oblong-lanceolate, regularly cuspidate serrate, venation strict-pinnate .. .. .. *Q. incana.* *Q. glauca.*

(i) Serrate to the base, laterals 12–20 pairs, generally 15–17 .. .. *Q. incana* (4)

(ii) Serrate from the middle, laterals 10–14 pairs generally 12–13 .. .. *Q. glauca* (5)

II. Leaves elliptic-oblong, ovate, oval-oblong, or ovate-lanceolate, entire or coarsely spinous-toothed, venation pinnate or pinnate-looped .. *Q. dilatata*
Q. semecarpifolia
Q. Ilex.

(iii) Venation conspicuously pinnate-looped, laterals 8–12 pairs, generally 10, thin and fine, tertiaries and finer reticulations conspicuous .. .. *Q. dilatata* (2)

(iv) Venation pinnate, laterals 4–6 pairs, upper 3 or 4 secondaries in toothed leaves form a sort of corymb, laterals intermediate in thickness between *Q. dilatata* and *Q. semecarpifolia* and tertiaries and finer reticulations somewhat conspicuous .. .. *Q. Ilex* (3)

(v) Venation faintly pinnate-looped, immersed, laterals 8–9 pairs and thickest of all species, tertiaries and finer reticulations mostly obscure .. .. .. *Q. semecarpifolia* (1)

(1) *Quercus semecarpifolia* Smith.

(The 'Karshu' Oak)

(Plate XV, Figs. 1–7 and Pl. XVI, Figs. 9, 10)

The fossil leaves figured here are of different sizes and also show a good deal of variation in shapes of the lamina, which may be elliptic-oblong, ovate, oblong-ovate or obovate in outline. Apex may be acute or rounded. Base is usually narrower, which may be tapering or rounded; in one specimen (Pl. XV, Fig. 4) it is slightly cordate. The margins are entire. (In some living leaves and a few badly preserved fossil leaves not figured here, the margins are cuspidately serrate varying from one tooth to an entirely toothed margin). A small thick petiole measuring about ·5 inch in length is preserved in one specimen (Pl. XV, Fig. 1).

The venation is strict-pinnate and reticulate. A strong midrib runs in the lamina making a deep groove in some (Pl. XV, Figs. 2, 4), which are evidently impressions from the lower surfaces of leaves; in others (Pl. XV, Figs. 1, 7), which are probably impressions from the upper surface, the midrib does not leave a groove. Usually it runs straight in the lamina gradually thinning out towards the apex and in figured specimens it seems to divide the lamina into slightly asymmetrical halves. 6 to 8 secondaries, which are about half as thick as the midrib, diverge from it on either side at different angles; the latter varying from obtuse to slight obtuse and sometimes to a

little acute angle in the figured specimens. Generally the lower and the upper secondaries come off at almost the same angles but occasionally one or two upper pairs of laterals may depart at slightly acute or open angles than the lower. The secondaries may fork either near the margins or far below in the lamina (Pl. XV, Figs. 1, 3); but sometimes they may curve upwards and end in the margins without forking (Pl. XV, Fig. 4). The forking is mostly indistinct but in some laterals (see Pl. XV, Fig. 1) it is very distinct and characteristic. Tertiaries are obsolete in the fossils, which are impressions from the upper surfaces, and they are very faintly visible in those, which represent the impressions from the lower surface. They seem to be arising at right angles from the secondaries (Pl. XV, Figs. 4, 5). These anastomose to form more or less cross-ties in the area between the two laterals (Pl. XV, Fig. 4). Finer reticulations are not visible in any of the specimens.

Our fossil leaves are identical in all respects with living leaves of *Quercus semecarpifolia* Smith., a large evergreen tree of the Western Himalayas, which is known in these regions as "Karshu oak" or "Kharshu" in vernacular language.

Number of specimens—About one hundred.

Occurrence—Laredura at 6,000–6,500 ft., Dangarpur at 6,300 ft. and Gogajipathri, at 8,800 ft., in the Pir Panjal Range, Kashmir.

Collectors—H. de Terra, B. Sahni, R. R. Stewart and G. S. Puri.

Registered Nos. of figured specimens: Pl. XV, Fig. 1 = L 165; Fig. 2 = L 562; Fig. 3 = L 590; Figs. 4, 5 = L 624/3; Fig. 6 = L 184; Fig. 7 = Loc. 1D/26; Pl. XVI, Fig. 9 = L 521; Fig. 10 = L 344.

(2) *Quercus dilatata* Lindl.

"The Moru Oak"

(Plate XVI, Figs. 11–17; Pl. XVII, Figs. 19, 20)

The fossil leaves belonging to this species are usually oblong, oblong-ovate or elliptic-oblong in outline. They vary a good deal in size ranging from 1·1 by ·55 inch (see Pl. XVII, Fig. 19) to 2·8 by 1·2 inches (Pl. XVI, Fig. 12). Base may be rounded, ovate or narrowed and oblique. Apex is always acute. The margins are entire, or coarsely cuspidate-serrate (Pl. XVI, Fig. 17).

In two specimens, which are counterparts, there occur on the surface a few insect galls (?), which are seen in the natural size photographs as raised dots (Pl. XVI, Fig. 15) and hollow cavities (Pl. XVI, Fig. 14) respectively.

Insect galls have been occasionally seen on living oak leaves and their occurrence in the fossil state is not very unusual.

The venation is pinnate-loped and reticulate. A fairly strong midrib runs straight in the lamina gradually thinning out towards the apex. It gives off at open angles on its either side in an alternate, opposite or sub-opposite manner 8–12 secondary ribs, which are finer than the midrib, but are fairly conspicuous. In one specimen (see Pl. XVI, Fig. 12) the laterals tend to run parallel to one another. In living leaves of this species the venation, though equally conspicuous on both surfaces, is not well marked out on either, hence it is not possible to say to which surface of the leaf the fossil impression belongs. The secondaries run straight to the margins and bifurcate beneath it into two small branches, which form semi-circular loops the convex sides of which are facing the margins. The adjoining loops tend to anastomose and form an infra-marginal vein running below and parallel to the margin. This looping is characteristic of this species, and is not found in any other oak. The tertiary ribs arise from the laterals on either side at right angles, and anastomose variously about

TABLE I

Characters	*Q. dilatata* Lind.	*Q. Ilex* L.	*Q. semecarpifolia* Sm.
1. Margins ..	Thin and not recurved	Thick and mostly recurved	Thick but very slightly recurved
2. Venation ..	Equally well marked on both surfaces and prominent in the fossils	Well marked on the lower surface only and mostly obscure in fossil leaves	Well marked on the lower surface only and obscure in fossils
3. Thickness of laterals	Thinnest	Intermediate between the three	Thickest of the three oaks
4. No. of laterals	8—12 pairs	4—6 pairs	6—8 pairs
5. Nature of laterals	They bifurcate under the margins and the branches form very distinct and characteristic loops, which join to form an infra-marginal vein, running below and parallel to the margins	Some bifurcate into thick branches, which do not form loops, but end in the margins either in the marginal teeth (if they are serrate) or in the entire margins	Rarely bifurcate into thick branches. Sometimes the laterals curve upwards to form indistinct loops relatively closer to the margins
6 Tertiaries ..	Form fine cross-ties or large rectangular meshes	Form large rectangular meshes	Form indistinct cross-ties or large meshes
7, Finer reticulations	Distinct	Slightly distinct	Mostly obscure
8. Nature of the two surfaces in living leaves	Smooth on both surfaces	Smooth on the upper surface, but thickly covered with white fluffy hairs on the lower surface. Hairs are large and stellate and they do not fall off in older leaves	Smooth on the upper surface but thinly covered with long hairs on the lower surface. Hairs fall off in older leaves

midway in the area enclosed by the two laterals to form large rectangular meshes, which are seen prominently in some specimens. Finer reticulation is also clearly seen in the enlarged photographs.

Our fossil leaves are identical in all respects with *Quercus dilatata*, the second most common Himalayan species, which occurs in a well-defined elevation zone lying intermediate with the zones of *Q. semecarpifolia* and *Q. incana*.

Some of the fossil leaves, which are not well preserved are likely to be confused with leaves of either *Q. Ilex* or *Q. semecarpifolia*, therefore, striking features of these three species are given in Table I in a tabular form.

Number of specimens.—About one hundred.

Occurrence.—Laredura at 6,000–6,500 ft., Dangarpur at 6,300 ft., Gogajipathri at 8,800 ft., in the Pir Panjal Range, Kashmir.

Collectors.—H. de Terra, B. Sahni, R. R. Stewart and G. S. Puri.

Registered Nos. of figured specimens.—Pl. XVI, Fig. 11 = L416, Figs. 12, 13 = L168, Fig. 14 = L253*a*, Fig. 15 = L253*b*, Fig. 16 = L523, Fig. 17 = L692; Pl. XVII, Fig. 19 = L492, Fig. 20 = L453.

(3) *Quercus Ilex* L.

"The Holly Oak"

(Plate XV, Fig. 8, Pl. XVI, Fig. 18 and Pl. XVII, Figs. 21–25)

The fossil leaves of this oak like other species of *Quercus* also vary a good deal in shape, size and nature of margins. The shape of leaf lamina is usually oblong, however, slight variations from the type are not infrequently met with. The size varies from ·8 × ·6 inch (Pl. XVI, Fig. 18) to 2·6 × 1·5 inches and even larger leaves are also found. Apex may be acute or broad and somewhat obtuse bearing a sharp pointed marginal tooth. Base is mostly broad, often rounded or subcordate. The margins are very variable, they may be entire, half serrate or coarsely spinous—toothed almost to the base. The teeth may be long, sharply pointed or small and blunt.

The venation is strict-pinnate and reticulate. Midrib is usually stout and thick and it has left a fairly deep groove in some leaves (Pl. XVII, Fig. 25). which seem to be impressions from the lower surface of the leaf. Plate XVII, Fig. 21 is an impression from the upper surface of a leaf. The midrib mostly runs straight in the lamina, but it may follow a zigzag course (Pl. XVII, Fig. 21). Four to six secondaries, which are about half as thick as the midrib, arise from it on either side at acute angles. The angle of origin of

the upper and the lower pairs of laterals in the same specimen may be slightly different. The lower secondaries diverge at open angles but the upper two or three pairs may arise at acuter angles. The laterals usually run straight to the margins and end in the marginal teeth in serrate leaves, but in non-serrate leaves they end in the entire margins. Some laterals, usually the upper one or two pairs, fork once or twice at different distances from the midrib. Tertiary ribs are generally not well preserved except in one specimen (Pl. XVII, Fig. 21), a part of which is enlarged to five diameters in the photograph (Pl. XVII, Fig. 22). They arise from the laterals at straight angles and anastomose among themselves variously to form large rectangular meshes. The tertiaries enclose a fine network of rectangular or pentangular meshes, which constitutes the finer reticulations.

Living or fossil leaves of *Quercus Ilex* L. have cuspidate serrate margins like *Ilex dipyrena* Wall. and *Acanthus ilicifolius* Linn. and they may be confused with these species. But a closer examination reveals important differences, which are brought out in the following table:—

Characters	*Q. Ilex* L.	*Ilex dipyrena* Wall.	*Acanthus ilicifolius* L.
1. Shape ..	Oblong or elliptic oblong	Lanceolate	Lanceolate
2. Venation ..	Fairly prominent	Rather obscure	Comparatively quite obscure
3. Midrib ..	Thick	Comparatively much thicker	Comparatively much thicker
4. Laterals-their nature and angle of divergence	Prominent and mostly forking ; acute	Less prominent and not forking ; obtuse, almost right angle	Least prominent and not forking ; obtuse like *Ilex*
5. Finer reticulations	Clear in the form of a uniform net-work of small-oval or rectangular meshes	Comparatively much less clear, consisting of wide irregular meshes	Obsolete

Our fossil leaves are identical in all respects with *Quercus Ilex*, an evergreen Himalayan shrub popularly known as "holly oak" on account of its spinous toothed leaves, which superficially resemble *Ilex* (see the above table).

Number of specimens.—About a hundred.

Occurrence.—Laredura at 6,000–6,500 ft., Dangarpur at 6,300 ft. and Gogajipathri at 8,800 ft.

Collectors.—H. de Terra, B. Sahni, R. R. Stewart and G. S. Puri.

Registered Nos. of figured specimens.—Pl. XVI, Fig. 18=L407; Pl. XVII, Figs. 21, 22=L517, Fig. 23 = L2, Fig. 24=L605, Fig. 25 = L700.

(4) *Quercus incana* Roxb.

"The Ban Oak"

(Plate XVII, Fig. 26 and Pl. XVIII, Figs. 33–34)

The fossil leaves described under this species are generally lanceolate or ovate-lanceolate in outline but like other oaks their shape and size may vary to a slight extent; the margins are invariably regularly closely cuspidate serrate. The marginal teeth, which vary in number from 11 to 14 on either side of the margin, are usually long and sharp with their sharp edges always directed upwards.

The venation is strict-pinnate and reticulate. A thick and prominent midrib, which arises from the base of the leaf, follows a slightly curved course and divides the lamina into unequal halves. 15–17 secondaries, which are almost half as thick as the midrib, diverge from it on either side at angles of 115 to 120 degrees in a sub-opposite, opposite or alternate manner. The lower 3 or 4 pairs of secondaries generally depart at somewhat obtuse, or less acute angles than the upper pairs which arise at sharply acute angles. The secondaries are equidistant; they run parallel to one another and ultimately end in the marginal teeth. In the basal part of the leaf, where there are no teeth they end in the entire margins; the number of the secondaries corresponds to the number of teeth in the serrate part of the leaf. Tertiaries are not well preserved in the figured specimens but in some unfigured fragmentary leaves they form cross-ties in the area between the two secondaries. Finer reticulation is not well preserved in any of the figured specimens.

Our fossil leaves are identical in all respects with *Quercus incana*, a moderate sized evergreen oak, which is one of the commonest trees of the Western Himalayan temperate forests.

Number of specimens.—About fifty.

Occurrence.—Liddarmarg at 10,600 ft., and Botapathri at 9,500 ft., in the Pir Panjal Range, Kashmir.

Collectors.—C. S. Middlemiss, H. de Terra, B. Sahni and G. S. Puri.

Registered Nos. of figured specimens.–Pl. XVII, Fig 26=B.1 Pl. XVIII, Fig. 34=B.2. Pl. XVIII. Fig. 33=K14/948 B.

(5) *Quercus glauca* Thunb.

(Plate XVIII, Figs. 28–32)

The leaves described below are oblong-lanceolate in shape with a narrowed base and acute apex. Leaf lamina, which is broadest in the middle,

becomes gradually narrowed towards base and apex. The margins are cuspidate serrate from the middle of the leaf, the lower part being entire. The marginal teeth, which are somewhat conical in shape, are small and blunt and their number on either side of the margin varies from 7–8.

The venation is strict-pinnate and reticulate. A strong midrib, which has left a fairly deep groove in some specimens (see Pl. XVIII, Fig. 31) that are evidently impressions from the lower surface of the leaves, runs in the lamina thinning out towards the apex. 11–12 secondaries which are about half as thick as the midrib, diverge from it on either side at angles of about 115–20 degrees. They arise from the midrib in an alternate manner and run straight in the lamina parallel to one another. These, too, like the midrib, have left deep grooves in the impressions. They end in the marginal teeth in the upper part of the leaf, but in the lower portion, where there is no tooth corresponding to a lateral, they end directly in the entire margins. Tertiary ribs are well marked and run conspicuously in the area enclosed by the two laterals. They arise from the laterals at right angles and meet about midway to form cross-ties, or large rectangular meshes (Pl. XVIII, Fig. 30). The finer reticulation is not well preserved in any specimen, but it seems to consist of a network of small meshes, which are oval or pentangular in shape.

Our fossil leaves are identical in all respects with *Quercus glauca* Thunb., a graceful Western Himalayan evergreen tree of the outer ranges.

Living and fossil leaves of *Quercus glauca* and *Quercus incana* being very similar in size, shape, venation, etc., are likely to be confused with each other. The following table is intended to give the differences by which the two species can be separated:—

Characters	*Quercus glauca* Thunb.	*Q. incana* Roxb.
I. *Living leaves*—		
(1) Size	4–7 by 1–3 inches.	Comparatively smaller 3–6 by 1–2 inches.
(2) Colour of the lower surface	Young leaves are covered on the lower surface by a few small silky hairs which fall off in older leaves. The hairs are simple and T-shaped.	White tomentose on the lower surface and thickly covered with long setllate hairs, which occur in roseltes. As many as 6-8 hairs may radiate from one hair base.
(3) Margins ..	Serrate from the middle, the lower part is entire.	Serrate almost to the base.
(4) Number of the laterals	10–14 pairs, generally, 12–13	12–20 pairs, generally 15
II. *Fossil leaves*	Of the above-mentioned characters it is clear that the first two cannot be made use of in the fossil impressions especially when the latter occur as fragments. The number of laterals also cannot be fully ascertained unless there is a complete leaf. So, the only reliable character which can help in identification of fossil leaves is the nature of the margins; and small fragments, provided their basal part is preserved can also be identified on the character of the margins.	

Number of specimens.—About fifty.

Occurrence.—Liddarmarg at 10,600 ft. in the Pir Panjal Range, Kashmir.

Collectors.—C. S. Middlemiss, G. S. Puri and H. de Terra.

Registered Nos. of figured specimens.—Pl. IV, Fig. 28 = K14/948.*a*26. Figs. 29–30 = Loc.3L73, Fig. 31 = K14/948.*a*8. Fig. 32 = K/4/948*a*.

Cupule:

Quercus sp.

(Plate XVIII, Fig. 35)

Pl. XVIII, Fig. 35 is a natural size photograph of an impression from the outer surface of the basal cup of an oak cupule, which has become flattened during fossilisation to assume a circular shape. A spherical dot in the middle of the specimen is the impression of the basal point with which the nut must have been attached to the branch. The scaly pattern seen in the photograph is impression from the outer surface markings of the cup.

Our specimen shows a striking resemblance to a cupule of *Quercus* but its specific determination cannot be attained due to the imperfectly preserved nature of the specimen.

Number of specimen.—One.

Occurrence.—Liddarmarg at 10,600 ft., in the Pir Panjal Range, Kashmir.

Collector.—C. S. Middlemiss, 1910.

Registered No. of figured specimen.—K14/948.

General Modern Distribution of the Genus *Quercus*

The genus *Quercus*, which contains about 300 modern species, is chiefly distributed at the present time in the north temperate regions and in tropical parts of mountainous Asia; in the tropics it extends from North America through the Mediterranean countries, namely, Southern Europe, the Caucasus, etc., into Asia and thence into the Malaya Archipelago. It is conspicuous by its absense from Central and South Africa and Australasia. Quite a few species are common in Afghanistan from whence they seem to have extended into the Himalayas. The Himalayan oaks are mostly hill species; a few are sometimes found in the plains, mostly as cultivated trees.

In India we have over 50 species of *Quercus*, some of them are of great importance in Indian forestry. Of these about 24 are confined mostly to Burma, though several of these extend into the neighbouring mountains

of Assam, Chittagong and also into Manipur. Some of the Burmese species are found in the Eastern Himalayas also and out of the ten species of *Quercus* known from this region the following four—*Q. lamellosa, Q. pachyphylla, Q. lineata* and *Q. spicata*—are the most important forest trees. In the Western Himalayas we have fives pecies, of which *Q. incana, Q. dilatata* and *Q. semecarpifolia* are by far the commonest and widely distributed forest tress; the other two species are *Q. Ilex* and *Q. glauca.* The Indian oaks, with an exception of one or two Burmese species, *e.g., Q. velutina* and *Q. semiserrata,* which are found at lower elevations, are all forest trees of the Himalayan regions and a few of these ascend to very high elevations and often merge into alpine forests or reach the upper limit of tree growth. A large number of the oaks are gregarious in their occurrence and usually form pure or mixed forests—either in association with conifers, or broad-leaved trees. Several species are well characterised in their altitudinal range and succeed one another regularly at different elevations in the Himalayas. The elevation zones are well defined in the case of the three North-Western Himalayan species, namely, *Quercus incana, Q. dilatata* and *Q. semecarpifolia,* which succeed one another from the lower to the higher altitudes in the above order.

The altitudinal zones of oaks generally coincide with those of the coniferous species, which also form characteristic belts both in the Western and Eastern Himalayas. The relation between the oak and coniferous forests in the Western and Eastern Himalayas is well brought out by the following table compiled by Champion (1936, p. 230).

Western Himalayas		Eastern Himalayas	
Broad-leaved forest	Coniferous forest	Coniferous forest	Broad-leaved fores:
1. *Rhododendron campanulatum* *Betula utilis*	*Abies Webbiana*	..	*Rhododendron* spp. *Betula utilis*
2. *Q. semecarpifolia*	*Abies Pindrow*	*Abies densa*	*Q. pachyphylla*
3. *Q. dilatata*	*Abies Pindrow* *Picea Morinda* *Cedrus Deodara*	*Picea spinulosa* *Tsuga brunoniana*	*Q. pachyphylla* *Q. lamellosa*
4. *Q. incana*	*Cedrus Deodara*	*Tsuga brunoniana*	*Q. lamellosa* *Q. lineata*
5. Sub-tropical	*Pinus longifolia*	..	..

Modern Distribution of the Fossil Species

Our fossil species are the most common forest trees of the Western Himalayas; they aremainly confined to these regions, but some of them have

a much wider distribution. The areas of their detailed distribution are given below:—

(i) *Quercus semecarpifolia.*—This species, at the present time, grows throughout the outer and moister ranges of the Temperate Himalayas from Bhutan westwards between the altitudes of 8,000 and 12,000 ft. It is particularly abundant on southern aspects; in the Kurram Valley it occurs at 9,000–11,000 ft; it also extends eastwards and occurs in K. & J. Hills (Assam) at 4,200 ft., Burma–Manipur frontier at 8,000–10,000 ft., and in China. Although it is a high level species, which usually occurs in regions of high snowfall and moderate monsoon rainfall, it may often descend to 6,500 ft., and at some places it grows abundantly below 8,000 ft. Towards the top of its altitudinal range it reaches the upper limit of tree growth and touches the treeless alpine meadows either directly or merges into the alpine forests of *Betula utilis* and *Rhododendron* sp. In the dry and arid valleys of the inner Himalayas it is unknown. It forms pure forests in its well characterised zone and also occurs abundantly in the coniferous forests of *Picea Morinda, Abies Webbiana, Taxus baccata* or even *Pinus excelsa.* It also forms large forests in association with deciduous dicotyledonous trees and occurs plentifully in the broad-leaved forests composed of *Pyrus lanata, Prunus Padus, Acer Cæsium, Juglans regia,* etc., towards the lower limit of its zone; at higher elevations it is associated with *Betula utilis, Rhododendron* sp. and *Abies Webbiana.*

A rich shrubby undergrowth composed of *Rosa macrophylla, Rubus niveus, Salix elegans, Viburnum stellulatum, V. cotinifolium, V. fætens, Lonicera quinquelocularis, Strobilanthes Wallichii,* etc., usually occurs in the forest of *Quercus semecarpifolia.*

Its distribution in the north-west and central Himalayas is not a continuous one and available data from the authentic sheets of the Herbaria show that it grows in the Kurram Valley, Hazara, Bashahr, Deoban, Jaunsar, Kulu, Chamba, Dharmsala, Naini Tal, Almora District and in K. & J. Hills (Assam).

The Kashmir distribution of the species with which we are specially concerned here is confined to the outer ranges; it grows in the Kishenganga Valley at 9,000 ft., also in Keran and Ramban. It is interesting to note that it has never been seen growing in the Kashmir Valley, the northern slopes of the Pir Panjal Range and the southern slopes of the Main Himalayas.

In the "western temperate broad-leaved forests" it forms pure forests locally but is mostly associated with other broad-leaved trees, *e.g.*, at Dhakuri

in the Central Almora division at an altitude of 9,500 ft., it occurs with *Betula alnoides, Pyrus lanata, Quercus dilatata, Acer Cæsium, Abies Pindrow, Meliosma dilleniæfolia, Pyrus foliolosus, Rhododendron arboreum, Betula utilis, Rosa macrophylla, Salix elegans, Viburnum fætens,* etc. (Champion, 1936, p. 236). At another place at an altitude of 11,000 ft., in the Garhwal Himalayas the common associates of *Q. semecarpifolia* are *Abies Pindrow, Rhododendron arboreum, Prunus Padus, Ilex dipyrena, Taxus baccata, Acer* spp., *Rosa serica, Rosa macrophylla, Cotoneaster acuminata, Salix elegans, Viburnum cotinifolium, V. stellulatum, V. fætens,* etc. (Champion, *loc. cit.*, p. 236).

In the " western mixed coniferous forests" it grows according to Champion (*loc. cit.*, p. 242), in Deoban, Chakrata at an altitude of 8,000 ft., in association with *Abies Pindrow, Picea Morinda, Euonymus lacerus, Rhamnus purpurea, Meliosma dilleniæfolia, Rosa macrophylla, Lonicera angustifolia, Viburnum stellulatum, V. cotinifolium, Hedera, Vitis himalayana,* etc., etc. It also grows in the spruce deodar belt of the Sutlej Valley; here its associates are *Picea, Cedrus, Abies Pindrow, Pinus excelsa, Q. dilatata, Q. incana, Acer acuminatum, A. Cæsium, A. pictum, Euonymus lacerus, Taxus baccata, Betula alnoides, Viburnum nervosum,* etc., etc. (Champion, *loc. cit.*, p. 242).

In the "western oak-fir forest", which is represented at Deoban in the Chakrata division, Jaunsar, it occurs with *Abies Pindrow, Picea Morinda, Q. dilatata, Acer Cæsium, Ilex dipyrena, Rosa macrophylla, Rubus niveus, Lonicera angustifolia, Viburnum fætens, Salix elegans, Rhamnus purpurea,* etc., etc. (Champion, *loc. cit.*, p. 245). At Pulga in the Parbatti Valley (Kulu) it occurs at an atitude of 8,500–9,000 ft. with *Abies Pindrow, Pinus excelsa, Picea Morinda, Acer* sp., *Taxus baccata, Corylus colurna, Pyrus lanata, Rosa macrophylla, Viburnum fætens, Berberis* sp., *Indigofera gerardiana,* etc., etc. (Champion, *loc. cit.*, pp. 246–47).

Quercus semecarpifolia reaches the upper limit of tree growth and is freely met within the Alpine forests, *e.g.*, in the "Alpine fir-birch forests" of the North Garhwal it occurs with *Abies Webbiana, Betula utilis, Pyrus foliolosa, Rhododendron campanulatum, Cotoneaster acuminatum, Rosa serica, Lonicera* spp., *Rubus niveus,* etc., etc. (Champion, *loc. cit.*, p. 270).

(ii) *Quercus dilatata* Lindl.—This species called "Moru" in the vernacular, is perhaps the largest of the Western Himalayan oaks and occurs from Nepal westwards at the altitude of 7,000 to 9,000 ft. Although it grows gregariously in all situations it seems to prefer cool and moist localities and is by far the commonest on northerly aspects; temperate conditions comprising a heavy winter snowfall and a good rainfall are congenial for

its growth. In cooler places it often descends as low as 5,000 ft., but far west in the Kurram Valley on Safedkoh and in Afghanistan it commonly ascends to 10,000 ft. It grows in the Kagan Valley and is a common tree in the Murree Hills, mostly occupying northern slopes. In Chitral it occurs in cooler places at 8,900 ft.

In Kashmir, though it is common in the Kishenganga Valley, the Lower Jhelum Valley, Keran, Kishtwar, Marwa Dachhan, Muzaffarabad, Ramban and Udhampur, it has never been found in the Valley proper, the southern slopes of the Main Himalayas or the northern slopes of the Pir Panjal Range.

It occurs in the outer ranges from Jaunsar to Kulu, at Kangra, Mussoorie, Naini Tal and Tehri Garhwal.

The Moru oak forms a well-defined zone, which is intermediate in altitude between the elevation zones of *Quercus semecarpifolia* and *Q. incana.* It occurs gregariously in pure patches or forms mixed forests with conifers in the Western Himalayas and with broad-leaved trees in the Central Himalayas. The commonest conifer occurring with it is *Picea Morinda,* though *Pinus excelsa, Cedrus Deodara* and *Abies Pindrow* are also frequently associated with it; it is often accompanied by *Taxus baccata.* Amongst the commonest broad-leaved trees occurring in the *Q. dilatata* forests we have several species of *Acer—Acer Cæsium* and *A. pictum* being the most common – *Ulmus Wallichiana, Prunus Padus, Cornus macrophylla, C. capitata, Euonymus pendulus, E. tingens, Juglans regia, Corylus colurna, Betula alnoides,* and *Populus ciliata*; shrubby plants of *Viburnum, Lonicera, Rubus, Rosa, Indigofera, Desmodium,* etc., are also present as an undergrowth.

Although the altitudinal range of the "Moru" oak is fairly well defined, *Quercus semecarpifolia* and *Q. incana* frequently merge into its zone and the three are often found growing together. Such an association is described by Champion (*loc. cit.*, p. 235) from Garhwal, where the associated flora includes *Betula alnoides, Carpinus viminea, Acer Cæsium, Machilus Duthiei, Euonymus pendulus, Ilex dipyrena, Rhamnus purpurea, Eurya acuminata, Buxus Sempervirens, Litsæa umbrosa, Pieris ovalifolia, Staphylea Emodi,* etc., etc.

Quercus dilatata occurs commonly in the "western temperate broad-leaved forests" and often forms pure patches usually growing with one or more species of *Acer.* In the "*Q. dilatata—Acer* forests", which are represented at Bhatkot in the Central Almora division, it occurs with *Q. incana, Abies Pindrow, Taxus baccata, Euonymus pendulus, Ilex dipyrena, Rhamnus purpurea, Eurya acuminata, Rhododendron arboreum, Meliosma dilleniæfolia, Cedrella serrata, Pyrus lanata, Pieris ovalifolia, Machilus*

Duthiei, Fraxinus micrantha, Betula alnoides, Rosa macrophylla, Myrsine semiserrata, Berberis aristata, Hedera Helix, etc., etc. (Champion, *loc. cit.*, p. 234).

Towards the upper limit of its range it merges into the zone of *Quercus semecarpifolia* and occurs in the "Kharshu" oak forest of Dhakuri in Central Almora division (Kumaon) at an altitude of 9,500 ft.

In the moist Deodar forests, which are a part of the western temperate coniferous forests, it occurs in the Kagan Valley (Hazara) with *Cedrus Deodara, Pinus excelsa, Parrotia jacquemontiana, Viburnum nervosum*, etc. (Champion, *loc. cit.*, p. 240).

In the "western oak-fir forest" of Deoban, Chakrata, it occurs with *Q. semecarpifolia, Acer Cæsium, Euonymus lacerus, Ilex dipyrena, Rosa macrophylla, Rubus niveus, Lonicera angustifolia, Abies Pindrow, Picea Morinda*, etc. (Champion, *loc. cit.*, p. 245).

(iii) *Quercus Ilex* L., which in India is mainly confined to the inner dry tracts of the Himalayas, is an evergreen shrub of a wide distribution occurring in the Mediterranean region from southern France and Spain eastwards through Italy, Morocco, Algeria, Tunis, and extends into India through Greece and Afghanistan; the arid aspects of the hills and drier localities provide most congenial habitats for its growth.

In India it occurs in dry temperate mixed evergreen forests, which are represented in the inner dry valleys of the Himalayas above the altitude of 5,000 ft. It ascends to various elevations in different places, thus on the Sulaman range it grows at 5,000–6,000 ft., in the Kurram Valley at 6,500-9,000 ft., in Chitral at 6,000–8,500 ft., in the upper dry valleys of the Jhelum, Chenab, Ravi and Sutlej at 4,400–8,500 ft., and in the Kagan Valley at 4,500–6,000 ft. It often descends to lower altitudes and occurs at 3,500 ft., in the hills lying north of the Peshawar Valley.

In Kashmir the species is common in the lower part of the Kishenganga Valley at the altitude of 3,500–7,000 ft., and also occurs in Keran, Kishtwar, Marwa Dachhan, Muzaffarabad and Ramban; it always occupies hotter aspects.

The common associates of this oak in the Kagan Valley, where it is gregarious, are *Fraxinus xanthoxyloides, Daphne oleoides*, and *Cedrus Deodara*, but in drier regions *Pinus gerardiana* is more frequent; it mostly occurs with *Cedrus Deodara* but in Chitral *Pinus excelsa* is associated with it.

In the "dry temperate mixed evergreen forests" of Kilba, upper Bashahr division, *Quercus Ilex* occurs in association with *Pinus gerardiana, Cedrus*

Deodara, Acer pentapomicum, Celtis australis, Fraxinus xanthoxyloides, Rhus succedanea, Parrotia jacquemontiana, Olea cuspidata, Zanthoxylon alatum, Daphne oleoides, Rosa Webbiana, Lonicera angustifolia, etc., etc. (Champion, *loc. cit.*, p. 254).

In upper Kagan Valley, Hazara, it grows with *Juniperus macropoda, Fraxinus xanthoxyloides, Daphne oleoides, Rosa Webbiana, Ribes grossularia*, etc., etc.

(iv) *Quercus incana* Roxb.—The "Ban" oak grows gregariously often forming pure forests, chiefly in the outer ranges of the Western Himalayas westwards from Nepal at an altitude of 4,000–8,000 ft; in moist ravines and other cooler aspects it descends to lower elevations and grows in the Mothronwala Swamp (Dehra Dun) at 1,900 ft. It occurs at 2,300 ft., in the Kangra Valley, and has been seen growing in the Lower Beas Valley in Kulu at 3,000 ft. Towards the upper limit of its altitudinal range it is freely mixed with *Quercus dilatata*, but at lower elevations in moist ravines it is found with *Q. glauca*. Although the "ban" oak grows on all aspects it usually prefers cool and moist ravines. When it grows with other temperate species, the oak takes drier and warmer situations. A temperate climate with a good monsoon rainfall and moderate snowfall is favourable for its growth though it often ascends to regions of heavy snowfall.

Forests of *Quercus incana* are fairly common in the Western Himalayas and here the oak is usually associated with broad-leaved trees which may be *Rhododendron arboreum, Pieris ovalifolia, Populus ciliata, Ilex dipyrena, Cornus macrophylla, C. oblongum, C. capitata, Pyrus Pashia, Acer oblongum, A. pictum, Ulmus Wallichiana, Ficus nemoralis, Carpinus viminea, Alnus nepalensis, Betula alnoides*; in moist ravines there may occur *Machilus Duthiei, M. odoratissima, Phœbe lanceolata, Litsæa umbrosa*, etc., etc. At other places the "ban" oak occurs with the conifers, namely, *Cedrus Deodara* and *Pinus excelsa*; at lower elevations it also occurs with *Pinus longifolia*. The drier aspects of the "ban" oak forests are usually occupied by shrubby plants of *Berberis, Indigofera, Desmodium*, etc., but at other places we may have *Viburnum cotinifolium, V. stellulatum, Berberis nepalensis, Lonicera quinquelocularis, Myrsine semiserrata, M. africana, Desmodium tiliæfolium*, etc., etc.

In the "western temperate broad-leaved forests" *Quercus incana* is the chief broad-leaved tree and according to Champion (1936, pp. 232, 233) it grows at Binsar in the W. Almora division, at an altitude of 6,500 ft. in association with *Carpinus viminea, Cedrela serrata, Rhododendron arboreum, Pieris ovalifolia, Ilex dipyrena, Betula alnoides, Viburnum cotinifolium*,

Myrsine africana, Desmodium tiliæfolium, Hedera Helix, etc., etc. Similar species occur with it in Garhwal and also in the Sutlej Valley in the Punjab.

In the "western mixed coniferous forests", which are represented in the Sutlej Valley, it occurs in association with *Quercus dilatata, Q. semecarpifolia, Acer acuminatum, A. Cæsium, A. pictum, Betula alnoides* and several conifers, namely, *Picea, Cedrus, Taxus baccata, Abies Pindrow* and *Pinus excelsa* (Champion, *loc. cit.*, p. 242).

In the "lower blue pine forests" of Udhampur division (Kashmir) it occurs at an altitude of 5,500 ft. with *Pinus excelsa, Cedrus Deodara, Quercus dilatata, Populus ciliata, Rhus succedanea, Viburnum cotinifolium, V. nervosum, Myrsine africana, Lonicera quinqueloculari*s, etc. (Champion, *loc. cit.*, p. 262).

The "moist temperate seral type forests" of *Quercus incana* occur in western Almora Division at an altitude of 6,500 ft. and also in Naini Tal division at 6,500 ft. (Champion, 1936, p. 264).

It also occurs in the "western temperate coniferous forests" especially in the moist deodar belt, *e.g.*, in sample plots in lower Bashahr we have associated with oaks *Pinus excelsa, Rosa macrophylla, Berberis Lycium, Lonicera angustifolia, Hedera Helix*, etc. (Champion, *loc. cit.*, pp. 237–39).

In the "Himalayan subtropical pine forests" the "ban' oak according to Champion (*loc. cit.*, p. 207), occurs at Kangra (Punjab) at an altitude of 3,000–3,500 ft. in association with *Pinus longifolia, Pyrus Pashia, Rhododendron arboreum, Mallotus philippinensis, Woodfordia fruticosa, Rhamnus triquetra*, etc.

(v) *Quercus glauca* Thunb. occurs generally in the outer Himalayan ranges between the altitudes of 2,000–6,000 ft., also in the Khasi Hills (Assam) and extends to Japan. It occurs with *Q. incana* and *Albizzia stipulata* at 3,000 ft. in the Kangra Valley. In the Simla Hills it grows at 3,000–4,000 ft. and is found between 3,000 and 5,000 ft, in Kempti Falls in Mussoorie.

It is a tree of moist ravines and occurs more or less gregariously on the banks of streams, and other moist situations in the valleys. Its common associates in moist localities in the Western Himalayas are *Q. incana, Machilus odoratissima, Machilus Duthiei, Phœbe lanceolata*, and several species of *Litsæa*, etc. In the Kumaon Himalayas it occurs along streams in the forests of *Pinus longifolia*.

Although this oak usually does not form pure forests, it is often gregarious and occurs in an almost pure forest between Baijnath and Dhelu in the Mandi State, where at an altitude of 4,000–5,000 ft. It is associated with

Q. incana, Pieris ovalifolia, Pinus longifolia, Rhododendron arboreum, etc. It grows plentifully in moist ravines at Garhi Habibullah and forests of Khanpur and Siran at 4,000–5000 ft. and is usually accompanied by *Pinus longifolia.*

Its associates in the Beas Valley are *Q. incana, Pistacia integerrima, Pyrus Pashia, Olea cuspidata,* etc.

In Kashmir it is recorded from the outer ranges at Mirpur, Muzaffarabad and Ramban but it is conspicuous by its absence from the Valley proper and the northern slopes of the Pir Panjal Range or the southern slopes of the Main Himalayas.

Oak Forests of the Kashmir Valley during the Pleistocene

The northern slopes of the Pir Panjal Range, which present a striking panaroma of a luxuriant vegetation belonging to temperate species at the present time, were characterised by forests of oaks, laurels, figs, box, *Mallotus, Woodfordia,* etc., etc., during the early Pleistocene times. The five species of *Quercus,* which occur in a great abundance in the fossil beds at five different localities, namely, Laredura, Dangarpur, Gogajipathri, Botapathri and Liddarmarg, must have formed during the Pleistocene, extensive forests in the Valley, in pure patches, or in association with broad-leaved genera or conifers.

The physical features of the Valley, during the Pleistocene, we are told, were very different from now, when it was occupied by tropical species. The Pir Panjal Range, unlike its present chain of lofty mountains was then represented by a low ridge over which the water-laden monsoon winds of the south-east seas could deposit their moisture in the interior parts of the valley, which is now cut off from these winds as well as from the severe heat of the adjoining plains. The climate was then of a tropical nature comprising of two wet periods, namely, July to september (when there was plenty of rain due to monsoon) and Mid-March to Mid-April (when the moisture was derived from melting of snow) and two dry periods, *viz.,* Mid-April to the end of June and October to Mid-March. Such conditions are analogous to those that we find to-day in the outer Himalayan regions. For example, in the Murree Hills and Kulu the vegetation is largely composed of oaks (as chief trees), and other tropical species; and the Pleistocene flora of Laredura, Liddarmarg, Botapathri, Dangarpur and Gogajipathri was probably very much like what we find to-day in the Murree Hills and Kulu. The three species of oaks, namely, *Q. semecarpifolia, Q. incana* and *Q. dilatata* probably

occurred on the northern slopes of the low ridge of that time in well characterised zones succeeding each other from higher to lower altitudes in the order given above; *Q. Ilex* must have occupied the drier and hotter aspects and *Q. glauca* probably existed at lower elevations and might have even merged in the zone of *Q. incana*.

From the discovery of the fossil species of *Quercus* at different localities in the deposits of the Pleistocene age, it appears that they occurred in two sharply defined groups: the first group comprising of *Q. semecarpifolia*, *Q. dilatata* and *Q. Ilex* flourished at Laredura, Dangarpur and Gogajipathri; and the second group, which includes *Q. glauca* and *Q. incana* seems to have occurred at Liddarmarg and Botapathri. At the present time, although *Q. semecarpifolia*, *Q. dilatata* and *Q. incana* occur in well defined elevation zones, they frequently merge into the zones of one another; but the occurrence during the Pleistocene of the five species in the above-mentioned strict groups is really interesting and difficult to explain with the data available to us at the present time.

Among the conifers that might have occurred with *Q. semecarpifolia*, *Q. Ilex* and *Q. dilatata* at Laredura, Dangarpur and Gogajipathri we may mention *Pinus* spp., *Abies* sp., *Cedrus Deodara*, *Taxus* sp., etc. The broad-leaved species, which were associated with them at the above localities are *Betula utilis*, *B. alnoides*, *Alnus nitida*, *Viburnum cotinifolium*, *Lonicera quinquelocularis*, *Fraxinus xanthoxyloides*, *Acer* spp., *Rosa macrophylla*, *Hedera Helix*, *Cotoneaster* spp., *Berberis* spp., *Litsæa* sp., *Olea glandulifera*, *Engelhardtia Colebrookeana*, *Rosa Webbiana*, *Rubus* spp., *Woodfordia fruticosa*, *Rhus punjabensis*, *R. Cotinus*, etc.

The associates of *Q. incana* and *Q. glauca*, at Liddarmarg, were:— *Machilus Duthiei*, *M. odoratissima*, *Phœbe lanceolata*, *Litseæ lanuginosa*, *Acer oblongum*, *Ficus Cunia*, *Acer pentapomicum*, *Buxus Wallichiana*, *B. papillosa*, *Berberis* spp., *Cornus Capitate*, *Myrsina africana*, *M. semiserrata*, *Mallotus philippinensis*, *Rhamnus triquetra*, *Elæodendron glaucum*, *Rhamnus virgatus*, *Berchemia floribunda*, *Desmodium podocarpum*, *D. laxiflorum*, etc.

Summary

1. The genus *Quercus* is abundantly represented in Karewa flora by five well determined species, namely, *Q. dilatata*, *Q. semecarpifolia*, *Q. Ilex*, *Q. incana* and *Q. glauca* which form the greater part of the material collected by Middlemiss, de Terra, Sahni, Stewart and the author from the Lower Karewa lake deposits of the Pleistocene age exposed at Liddarmarg, Gogajipathri, Dangarpur, Laredura and Botapathri.

2. The oaks, which must have been dominant trees in the flora of the Kashmir Valley during the Pleistocene, are unrepresented in the modern vegetation of the valley and of the mountain slopes flanking it to the north-east and south-west. Three of the fossil species, namely, *Q. semecarpifolia*, *Q. dilatata* and *Q. incana* are at present very common in the outer ranges of the western temperate Himalayas, often forming pure forests or occurring with conifers, or broad-leaved trees in the well-defined elevation zones which succeed each other from the higher to the lower altitudes in the above order. Regions with heavy monsoonic rainfall and moderate snowfall (*Quercus semecarpifolia* reaches the upper limit of the tree growth) are congenial for their growth. *Quercus Ilex* is confined to the inner dry ranges of the Himalayas, whereas *Quercus glauca* occurs in the outer ranges at lower elevations.

3. The modern distribution of the five species is given in some detail and their present associates (conifers and broad-leaved genera) in different types of forests in the Himalayas are mentioned.

4. The significance of the occurrence of the oaks in the Kashmir Valley during the Pleistocene is discussed and it is concluded that the valley, at a time when the oak forests were flourishing on the northern slopes of the Pir Panjal Range must have had a tropical climate unlike the temperate conditions of the present day.

5. The abundance of oak leaves in these deposits supports the universally accepted theory of the Pleistocene uplift of the Himalayas.

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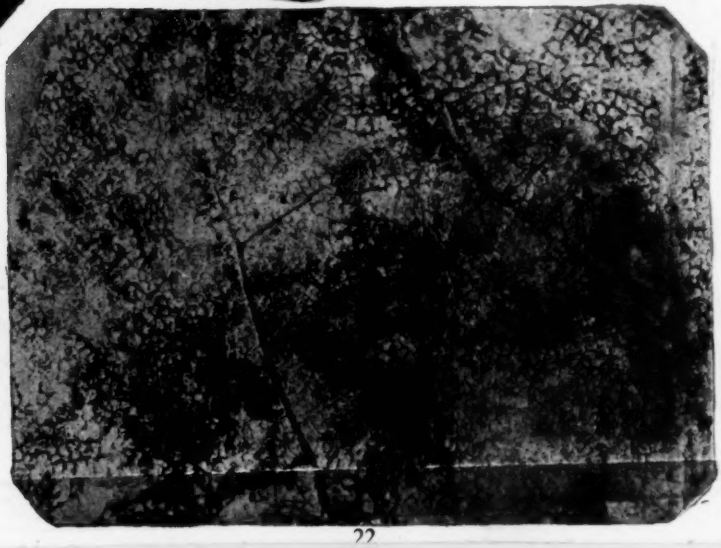

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Wodehouse, R. P., and H. de Terra "The Pleistocene pollen of Kashmir," *Mem. Conn. Acad.*, 1935, 9.

Explanation of Plates

The photographs in Plates XV–XVIII are from untouched negatives. The figured specimens except for Middlemiss's collection which is the property of the Geological Survey of India, are preserved in the University of Lucknow (Sahni collection).

Plate XV

Quercus semecarpifolia Smith.

Fig. 1. Leaf (impression of the upper surface). R. R. Stewart collection, L 165, Laredura. Nat. size.

Fig. 2 Leaf (impression of the lower surface). G. S. Puri collection, L 562, Laredura. Nat. size.

Fig. 3. Leaf impression. G. S. Puri collection L 590, Laredura. Nat. size.

Fig. 4. Leaf (impression of the lower surface). G. S. Puri collection, L 624/3 Laredura. Laredura. Nat. size.

Fig. 5. A small part of the leaf (Fig. 4) enlarged to show tertiary and finer reticulations. × *Ca.* 5.

Fig. 6. Leaf (impression of the upper surface). R. R. Stewart collection L 184. Nat. size.

Fig 7. Leaf (impression of the upper surface). H. de Terra collection, Loc. 1 D/26, Dangarpur. Nat. size.

Quercus Ilex L.

Fig. 8. Leaf (impression of the lower surface). R. R. Stewart collection, L 373, Laredura. Nat. size.

Plate XVI

Quercus semecarpifolia Smith.

Fig. 9. Leaves (impression of the lower surface). G. S. Puri collection, L 521, Laredura. × *Ca.* Nat. size.

Fig. 10. Leaf impression showing slightly arcuate laterals. R. R. Stewart collection, L 344, Laredura. Nat. size.

Quercus dilatata Lindl.

Fig. 11. Leaf impression. R. R. Stewart collection, L 416, Laredura. Nat. size.

Fig. 12. Leaf impression. R. R. Stewart collection, L 168, Laredura. Nat. size.

Fig. 13. A part of the leaf (Fig. 12) enlarged to show nature of the laterals: tertiary and finer reticulations. × *Ca.* 5.

Fig. 14. Leaf impression showing insect galls. R. R. Stewart collection, L 253*a*, Laredura. Nat. size.

Fig. 15. Counterpart of Fig. 14; the insect galls appear as raised dots. L 253*b*. Nat. size.

Fig. 16. Leaf impression. G. S. Puri collection, L 523, Laredura. Nat. size.

Fig. 17. Two spring leaves with serrated edges, a part of the one is lying on the other. G. S. Puri collection, L 692, Laredura. Nat. size.

Quercus Ilex L.

Fig. 18. A very small leaf (impression of the lower surface). R. R. Stewart collection, L 407, Laredura. Nat. size.

PLATE XVII

Quercus dilatata Lindl.

Fig. 19. A very small leaf impression. R. R. Stewart collection, L 492, Laredura. Nat. size.

Fig. 20. Leaf impression of medium size. R. R. Stewart collection, L 453, Laredura. Nat. size.

Quercus Ilex L.

Fig. 21. Leaf (impression of the upper surface) showing irregularly toothed margin and peculiar bifurcation of upper laterals. G. S. Puri collection, L 517, Laredura. Nat. size.

Fig. 22. A part of the leaf (Fig. 21) enlarged to show tertiary and finer reticulations × *Ca.* 5.

Fig. 23. Leaf impression. B. Sahni collection, L 1, Laredura. Nat. size.

Fig. 24. A coarsely spinous toothed leaf (impression of the lower surface). G. S. Puri collection, L 605, Laredura. Nat. size.

Fig. 25. Leaf impression with two coarsely spinous teeth. G. S. Puri collection, L 700, Laredura. Nat. size.

Quercus incana Roxb.

Fig. 26. Leaf (impression of the lower surface). G. S. Puri collection, B 1, Botapathri. Nat. size.

PLATE XVIII

Quercus incana Roxb. or *Q. glauca* Thunb.

Fig. 27. Leaf (impression of the upper surface) representing the upper part only. Middlemiss collection, G.S.I. No. K 14/948 *a* 9. Nat. size.

Quercus glauca Thunb.

Fig. 28. Leaf (impression of the lower surface). Middlemiss collection, G.S.I. No. K 14/948 *a* 26. Nat. size.

Fig. 29. Leaf impression showing the margin (serrate from middle). H. de Terra collection, Loc. 3 L 73, Liddarmarg. Nat. size.

Fig. 30. A small part of the leaf (Fig. 29) enlarged to show a lateral ending in a marginal tooth, tertiary and finer reticulations. × *Ca.* 5.

Fig. 31. Leaf (impression of the lower surface). Middlemiss collection, G.S.I. No. K 14/948 A, Liddarmarg. Nat. size.

Fig. 32. Leaf (impression of the lower surface). Middlemiss collection, G.S.I. No. K 14/948 *a*, Liddarmarg. Nat. size.

Quercus incana Roxb.

Fig. 33. Leaf (impression of the upper surface). Middlemiss collection, G.S.I. No. K 14/948 B, Liddarmarg. Nat. size.

Fig. 34. Leaf impression. B. Sahni collection, B 2, Botapathri. Nat. size.

Quercus sp.

Fig. 35. Cupule (impression of the outer surface). Middlemiss collection, K 14/948 *a* 8, Liddarmarg. Nat. size.

838-48. Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore,

SOME MACROSCOPIC PLANT-REMAINS REFERRED TO THE BETULACEÆ FROM THE KAREWA DEPOSITS OF KASHMIR

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Received July 5, 1945

(Communicated by Professor Birbal Sahni, F.A.SC., F.R.S.)

(With Three Plates)

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Introduction

The material described in this paper was collected partly by Dr. R. R. Stewart in 1935 and 1936 and partly by the author in 1939 and 1941 from the Lower Karewa deposits of Kashmir, which are exposed at an altitude of 6,000 ft. near the village of Laredura(Lat. 34° 7′; Long. 74° 21′)and in a grassy meadow that lies at an elevation of 9,000 ft. on the right bank of Ningal Nullah (Lat. 34° 4′; Long. 74° 19′). In addition, this paper also describes two leaves of *Alnus nepalensis* from a collection of fossil plants made in 1910 by the late Mr. C. S. Middlemiss from Liddarmarg (Alt. 10,600 ft.; Lat. 33° 48′; Long. 74° 39′), Pir Panjal, which was lent to the author by the Director, Geological Survey of India.

The majority of the fossils excepting a few female cones referable to *Betula* and *Alnus*, and four other specimens, which favourably compare with pieces from the bark of *Betula*, are leaf-impressions, most of which on account of their well preserved venation could be specifically determined with the modern flora of the Himalayas.

The present paper was prepared under the guidance of Prof. B. Sahni, SC.D., F.R.S., to whom I am highly indebted for his invaluable help and stimulating criticism. I am also thankful to Dr. R. R. Stewart, Professor of Botany and Principal, Gordon College, Rawalpindi, for the invaluable help he gave me in identifying the fossils in his herbarium during the years 1937–39.

I must acknowledge with thanks the research grants from the Vice-Chancellor, University of the Punjab, and Principal of the Khalsa College, Amritsar, and a Research Fellowship from the Lucknow University to enable me to carry on my work at Lucknow.

For a loan of the Middlemiss's material and a photograph of a leaf of *Alnus nepalensis* I am thankful to the Director, Geological Survey of India. My wife has helped me in correcting the proofs.

Systematic List of the Species

Alnus nepalensis D. Don.
Alnus nitida Endl.
Betula utilis D. Don.
Betula alnoides Buch-Ham.
Betula sp. A.
Betula sp.
Alnus sp.
Betula sp.

Description

Order: Fagales

Family: Betulaceæ

The Betulaceæ is represented in the Karewa flora by a few specimens of female cones in addition to leaf impressions, which are identified with two distinct modern genera, namely, *Betula* and *Alnus*; besides these, the collections contain a few specimens of *Betula* bark as well. The two genera include four well determined species, two belonging to each genus, and one incompletely determined species of *Betula*; all of these are based on leaf fragments.

Leaves of the Betulaceæ, whether living or fossil, are not difficult to determine but to assign female cones to their respective species is a very hard task, especially when the latter occur as small fossil fragments. The modern female cones of the two species of *Betula* are distinguished from each other by the nature of fruiting bracts, which are deeply 3 lobed in *B. utilis* D. Don., whereas in *B. alnoides* Buch-Ham. the bracts are slightly two lobed. The size of the modern female cones of the two species of *Alnus* varies a good deal and the species can be separated from each other on this character alone; the cones measure 1–1·3 inches in length in *Alnus nitida* Endl., while their size in *Alnus nepalensis* D. Don. ranges between ·5 and ·8 inch.

Our fossil cones are not only fragmentary but are rather poorly preserved and do not show much structural detail. They occur in the clay in a longitudinal plane. Two cones of *Alnus*, which were slightly better preserved than the rest, were ground to show up some details of structure in a longitudinal section. One of them, being too brittle and soft, was partly lost in grinding while the second specimen was successfully ground to show up some structure, which is brought out in a photograph in Pl. XXI, Fig. 21.

In the year 1910 the late Middlemiss (1911, p. 122) recognised among his collections from Liddarmarg two leaves of *Alnus*, which have now been specifically determined by the author as *Alnus nepalensis* D. Don. The late Dr. S. K. Mukerjee (see de Terra and Wodehouse, 1935, p. 2) also recognised in de Terra's collections of 1932 a few leaves of *Alnus* from the same locality, which are also identified by the author with *A. nepalensis* (Puri, 1941, p. 8).

KEY TO THE GENERA

I. Leaves serrate, cones large and elongated .. .. *Betula* (i)
II. Leaves entire, cones small and oval .. .. *Alnus* (ii)

1. *Leaves*

(i) *Genus Betula* Linn.

This genus includes two distinct fossil species, the modern representatives of which occur at the present time in the Himalayas; there are two more leaf fragments, which are undoubtedly referable to this genus but they do not seem to match any modern species of *Betula* that grows to-day in the Himalayan region.

The cones could not be referred to either of the two modern species, on account of their fragmentary nature and the reasons outlined above.

In addition to leaves and cones a few specimens of thin outer coverings from the bark of *Betula* sp. are also described here. These specimens being badly preserved do not show any cellular structure; hence it has not been possible to identify them with any modern species of *Betula*, although they show a good deal of resemblance to *B. utilis*.

Key to the Species—

I. Leaves small, oblong ovate .. .. *B. utilis* and *Betula* sp. A.
(i) Secondaries fewer, 6–7 pairs, arising wide part .. *B. utilis* (1)
(ii) Secondaries, 10–11 pairs, closely situated .. *Betula* sp. A (3)
II. Leaf large, ovate, secondaries many, 18–19 pairs .. *B. alnoides* (2)

(1) *Betula utilis* D. Don.

(Plate XIX, Figs. 1, 2 and Pl. XX, Figs. 7, 8)

Plate XIX, Fig. 1 is a natural size photograph of a leaf fragment, which is about half of the leaf representing one side of the midrib only, the other part of the lamina being greatly damaged on the margin as well as apex and base. It is well preserved regarding details of venation and measures 1·34 inches by ·9 inch in size. The leaf lamina probably had an ovate outline with a broad base and acute apex. Comparison with a living leaf (Pl. XIX, Fig. 2) of this species shows that the above description of the fossil leaf is not far from satisfactory. A small curved petiole, which is ·3 inch long, is also preserved in the fossil. Another fragment assigned to this species is illustrated in a natural size photograph No. 7 on Plate XX. This, too, is equally badly preserved but shows an acute apex and serrate margin, for which it is figured here.

The venation is strict-pinnate and reticulate. A strong midrib runs in the fragment slightly thinning out in the upper part. 5 to 7 secondaries, which are almost half as thick as the midrib, arise on either side in an opposite, sub-opposite or alternate manner at irregular distances and diverge in the lamina making acute angles with the midrib. The lowermost secondary on the right-hand side in one fossil fragment (Pl. XIX, Fig. 1) gives off two small and thin branches. Two or three other basal secondaries also give off thin branches close to the margin and the latter, being as thin as the tertiary ribs get mixed and even lost in the meshes of the tertiary reticulations. The tertiaries are likewise thin, but conspicuous; they run irregularly in the area occupied by the two laterals forming large rectangular meshes, which are well brought out in a part of the leaf (Pl. XX, Fig. 7) enlarged to five diameters in another photograph (Pl. XX, Fig. 8). There is a finer reticulation, which consits of a network of small oval meshes; these being poorly preserved are not brought out satisfactorily in the photographs.

The leaf fragments are identical in all respects with modern leaves of *Betula utilis* D. Don. (see Pl. XIX, Fig. 2), a high level birch of the Himalayas, which is a common element of the modern alpine forests of Kashmir.

No. of specimens.—Ten only.

Occurrence.—Laredura, at 6,000 ft. and Ningal Nullah, at 9,000 ft., Pir Panjal, Kashmir.

Collectors.—R. R. Stewart, 1935, G. S. Puri, 1941.

Registered Nos. of figured specimens.—Pl. XIX, Fig. 1 = L 500; Pl. XX, Fig. 7 = N 7.

(2) *Betula alnoides* Buch.-Ham.
(Plate XIX, Figs. 3–4)

Plate XIX, Fig. 3 is a natural size photograph of a fossil leaf, which is almost complete and measures 3·6 inches long by 1·6 inches in the broadest part. The margins are serrate; teeth are small and blunt (Pl. XIX, Fig. 4). The leaf lamina, which had probably an ovate shape, gradually narrows from the middle upwards into an acute apex and downwards into a wedge-shaped base.

The venation is thin and fine, but it is fairly well preserved and conspicuous; it is strict-pinnate and reticulate. A fine midrib, which thins out still further in the upper part, gives off on its either side about 18 secondaries which are also fine and about half as thick as the midrib; they diverge from the midrib at acute angles and usually arise in an opposite manner becoming alternate in the upper part of the lamina. They run in the lamina parallel to one another and finally end in the marginal teeth. A number of much finer tertiary ribs arise from the secondaries to meet (with each other) about half way in the area enclosed by the two laterals to form cross-ties or large rectangular meshes, which are clearly brought out in a part of the leaf enlarged to five diameters in Pl. XIX, Fig. 4. The finer reticulation is in the form of small meshes, which are of different shapes and sizes.

The fossil leaf nicely matches in shape, margins and all other details of venation with modern leaves of *Betula alnoides* Buch-Ham. and in the absence of any difference between the two, the fossil is identified with this species.

No. of specimens.—One only.

Occurrence.—Laredura, at 6,000 ft., Pir Panjal, Kashmir.

Collector.—G. S. Puri, 1941.

Registered No. of figured specimen.— L 804/3.

(3) *Betula* sp. A
(Plate XX, Fig. 6 and Pl. XXI, Fig. 17)

The specimens described below are two almost complete leaves, both of which are slightly broken at apex; the smaller leaf measures 1·38 inches long by ·95 inch in the broadest part, which is about midway between base and apex. The leaf lamina is ovate-oblong in outline with a semi-cordate base and acute apex. The margins are closely sharp serrate; the marginal teeth are mostly thick and pointed but a few of these are further subdivided into smaller teeth and as a result of these the margins look irregularly biserrate (Pl. XXI, Fig. 17) at some places.

The venation is thin, but prominent; it is strict-pinnate and reticulate. A thin midrib arises from the base, and gradually thins out as it traverses the lamina towards the apex. 10–11 pairs of secondaries, which are not even half as thick as the midrib, diverge from it at angles of about 40 degrees in an opposite and a rarely alternate manner; they are equidistant and run parallel in the lamina, and end in the marginal teeth. The tertiaries form regular meshes, which are rectangular in shape; these are well preserved and clearly seen at some places in both specimens. The larger meshes of the tertiaries are occupied by a close network of still smaller meshes, which constitute the finer reticulations.

Our fossil leaf fragments undoubtedly belong to the genus *Betula*; however, they do not match leaves of any species of *Betula* represented in the present-day flora of the Himalayas and may constitute a new type, which has probably become extinct from this part of the world since the pleistocene. Although the differences from the modern species are fairly marked out to constitute our fossil a distinct new species, the author has preferred the conservative course and has described it here as *Betula* sp. A and its salient features are compared to the two modern species so as to bring out the differences clearly in the following table:—

Characters	*B. utilis* D. Don. (Pl. XIX, Figs. 1,2)	*B. alnoides* Buch Ham. (Pl. XIX, Figs. 3, 4 and Pl. XX, Figs. 7–8)	*Betula* sp. A (Pl. XX, Fig. 6 and Pl. XXI, Fig. 17)
1. Outline ..	Probably broadly ovate	Oblong ovate	Oblong
2. Base ..	Broad	Wedge-shaped	Semi-cordate
3. Margins ..	Sharply serrate	Bluntly serrate	Serrate and rarely biserrate
4. Secondaries:			
(i) their number ..	6–7 pairs	17–18 pairs	10–11 pairs and the broken part may have one or two pairs more
(ii) their nature ..	Thick and arise wide apart	Thin and arise closely situated	Much thinner and comparatively more closely situated

No. of specimens.—Two only.

Occurrence.—Laredura, at 6,000 ft., Pir Panjal, Kashmir.

Collectors.—G. S. Puri, 1941 and R. R. Stewart, 1936.

Registered Nos. of figured specimens.—Pl. XX, Fig. 6 = L 774/4; Pl. XXI, Fig. 17 = L 200.

(ii) *Genus Alnus* Linn.

Two species belonging to this genus are represented in the Karewa flora by a number of leaf fragments and a few specimens of female cones. The leaves are well preserved impressions, two of which are almost complete, but a few others are incomplete fragments. The cones, unlike the leaves, are rather poorly preserved, and do not show sufficient structural details to enable us to identify them with one or the other modern species. They were coated, when collected, with varnish and canada-balsam, which although obscured all structure, proved helpful in a way that the tissues of the cones became sufficiently hardened by impregnation of the balsam; one of them was successfully ground to show some internal structure in a longitudinal plane.

Key to the Species—

I. Leaf broadly ovate with acute apex, secondaries 10–12 pairs, not parallel; tertiaries form rectangular meshes .. *A. nitida* (1)

II. Leaf ovate-oblong with a more or less rounded apex, secondaries 16–17 pairs, parallel; tertiaries form conspicuous cross-ties .. .. *A. nepalensis* (2)

(1) *Alnus nitida* Endl.

(Plate XX, Figs. 12–13 and Pl. XXI, Fig. 14)

Plate XX, Fig. 12 is a natural size photograph of a fossil fragment, which is about three-fourth of the entire leaf representing the apical portion only, the basal part being missing. The specimen is poorly preserved except for the apical part, which shows all details of venation. It measures 3·6 inches long by 2·2 inches in the broadest part. The leaf lamina, which probably had a broadly ovate outline, gradually narrows upwards into an acute apex. From the shape of the fragment it seems that the leaf had probably a broad base, and comparison with living leaf of this species does not contradict this suggestion. The margins are entire. Plate XX, Fig. 13 illustrates another fragment, which is a counterpart from the tip of the larger fragment figured here.

The venation is pinnate-looped and reticulate. A strong midrib, which has left a fairly deep groove in the impression at some places, follows a zig-zag course in the lamina; it gradually thins out towards the apex and gives off 8 to 9 secondaries, which are about half as thick as the midrib; the laterals arise in an alternate manner at almost equal distances. The secondaries unlike the midrib run staright and tend to lie parallel to one another; they bifurcate in the lamina while still away from the margins and the two branches run wide apart and slightly curve upwards and downwards to anastomose with similar branches of the superior and inferior secondaries respectively, thus, a series of simple loops is formed beneath the margins (Pl. XXI, Fig. 14). From the convex side of the loops there arise 2 to 3 small, equally thick branches, which anastomose with one another to form an almost indistinct second series of angular loops that lies comparatively closer to the margins (Pl. XXI, Fig. 14). The midrib and the laterals have left fairly deep grooves in the leaf, which seems to be an impression of the lower surface. The tertiary ribs arise from the laterals as well as the midrib and anastomose midway in the lamina to form large rectangular or pentangular meshes (Pl. XXI, Fig. 14). The finer reticulation is very clear and it is composed of a network of small oval or rectangular meshes, which are occupied in the larger meshes of the tertiary reticulations.

Our fossils resemble living leaves of *Alnus nitida* Endl. in all details of venation and in the absence of any difference between the two the former are identified with this species.

Number of specimens.—Two only.

Occurrence.—Laredura, at 6,000 ft., Pir Panjal, Kashmir.

Collector.—G. S. Puri, 1939.

Registered Nos. of figured specimens.—Pl. XX, Fig. 12 = L 543; Pl. XX, Fig. 13 = L 544.

(2) *Alnus nepalensis* D. Don.

(Plate XX, Fig. 11 and Pl. XXI, Figs. 15, 16)

The fossil fragments described under this species are two leaf impressions, which are fairly well preserved and show details of venation clearly. They vary in size from 2·7 inches by 1·9 inches (in the almost complete specimen) to 3·2 inches by 2·25 inches (in the half complete leaf). Outline of the lamina is ovate in one and ovate-oblong in the other specimen. Base is narrowed and somewhat rounded. Apex is broken, but seems to have been acute, comparison with a living leaf of this species justifies this assumption. The margins are entire.

The venation is pinnate-looped and reticulate. A stout midrib runs in the lamina retaining almost the same thickness throughout. 8 pairs of laterals, which are half as thick as the midrib, arise in the fragment (Pl. XXI, Fig. 11) and there are 16 pairs in the complete leaf (Pl. XX, Fig. 16) ; they diverge from the midrib in an opposite, sub-opposite and, rarely in an alternate manner. Two or three lower pairs of laterals in one specimen (Pl. XX, Fig. 11) and almost all secondaries, excepting two or three pairs near the apex in the other leaf (Pl. XXI, Fig. 16), shoot out from the midrib at open angles, which almost become right angles in two or three lowermost laterals in the complete leaf. There is a good deal of difference in the angles at which the secondaries diverge in living leaves, but in all cases they run parallel and are constant in number. Similar variations are also seen in the fossil leaves described here. The laterals are more or less equidistant and run straight towards the margins keeping a uniform distance between them. On reaching the margins they suddenly curve upwards to anastomose with their superiors and form a continuous series of loops, which runs below and parallel to the margins. This inframarginal vein which is seen in the upper part on the left-hand side in one specimen (Pl. XX, Fig. 11) and towards the basal part on the left-hand side in the other (Pl. XXI, Fig. 16) is a characteristic feature of this species. The midrib and the secondaries have left fairly deep grooves in the fossils, which seem to be impressions of the lower surface. The tertiary ribs are prominent and form cross-ties in the area enclosed by two secondaries; these enclose a well preserved network of smaller meshes, which are of different shapes and slightly different sizes. The latter constitute a finer reticulation, which is clearly seen in a part of the leaf (Pl. XX, Fig. 11) enlarged in the photograph (Pl. XXI, Fig. 15) to five diameters.

The fossils nicely match in shape, size, margins and all details of venation with living leaves of *Alnus nepalensis* D. Don., and in the absence of any difference between the two they are identified with this species.

Number of specimens.—Five only.

Occurrence.—Ningal Nullah at 9,000 ft., and Liddarmarg, at 10,600 ft., Pir Panjal, Kashmir.

Collectors.—C. S. Middlemiss, 1910; R. R. Stewart, 1936 and G. S. Puri, 1941.

Registered Nos. of figured specimens.—Pl. XXI, Fig. 16= K 14/949 (i); Pl. XX, Fig. 11 = N. 120.

2. *Female Cones*

Betula sp.

(Pl. XX, Figs. 9, 10)

Two specimens (in counterparts) of female cones described here belong to the genus *Betula*. The specimens lie in the clay partly exposed in a roughly longitudinal section. They were painted with a thick rubber solution by Dr. Stewart in field immediately after they were collected. This treatment spoiled both specimens; and the rubber solution got so tenaceously stuck on the surface of the fossils that it could not be removed to clarify the obscured structure of the bracts, this has made it impossible to say whether they are three lobed or the lobes are two in number.

The fossil cone, which is almost complete, measures ·9 inch long by ·38 inch in the broadest part. It consists of a stout central axis, which slightly thins out towards the apical end. A large number of bracts, which are probably fairly strong, are compactly and closely arranged on it in a more or less spiral manner. This arrangement of the bracts on the axis is not very clearly seen in the photograph on account of the rubber solution but it is fairly clear in the specimen. The bracts arise from the axis at right angles; each bract is broader at the base but becomes narrowed towards the upper part which is seen towards the outer side in the photograph (Pl. XX, Fig. 10).

Occurrence.—Laredura at 6,000 ft. in the Pir Panjal Range, Kashmir.

Number of specimens.—Two only.

Registered No. of figured specimen.—L. 25.

Collector.—R. R. Stewart, 1935.

On account of their badly preserved nature structural details of the cones are not clear enough as to afford comparison with female cones of the two modern species of *Betula*. Parker (1918, pp. 492, 493) gives the following distinguishing features of female cones of the two living species of *Betula*:—

Characters	*B. utilis* D. Don.	*B. alnoides* Buch Ham.
1. Length ..	Not more than 1·5 inches	Often 2–3 inches long
2. Bracts ..	Deeply three lobed, lobes are linear oblong	3 lobed, central lobe large and oblong, the two lateral lobes are small tooth-like
3. Fruit ..	It is narrower than the bract	It is broader than the bract
4. Wing of the fruit ..	It is narrower than the nut	It is broader than the nut

The above-mentioned features, which have a diagnostic value in living cones, are not seen in the fossils; therefore, it is impossible to identify them with the help of these. An attempt to grind the fossil in order to get the cross-sectional view was not successful; hence they could not be specifically determined.

Alnus sp.

(Plate XXI, Figs. 20–22)

Plate XXI, Fig. 20 is a natural size photograph of a female cone of this genus. At first the cone did not show any structure but on grinding it in a longitudinal plane it showed a central axis on which several bracts are arranged in a spiral manner. Pl. XXI, Fig. 21, which is a five diameters enlarged photograph of the cone, does not show the structure very clearly. The bracts are not well preserved on the left-hand side of the axis in the specimen, and due to the badly preserved nature of the cone the structure of seeds cannot be ascertained.

It measures ·8 inch long by ·6 inch in the broadest part.

Occurrence.—Ningal Nullah at 9,000 ft., Pir Panjal Range, Kashmir.

Number of specimens.—Four only.

Registered Nos. of figured specimens.—N 230 and N 231 *a*.

Collector.—R. R. Stewart, 1936.

The fossil specimens resemble living female cones of *Alnus* and in the absence of any difference between the two they are identified with this genus. On account of the poorly preserved nature of the fossils their specific determination is not possible; however, the size of one specimen, which is probably a complete cone, agrees with living cones of *Alnus nepalensis* D. Don., which measure according to Parker (*loc. cit.*) from ·5 to ·8 inch long. The size of the second species, *viz.*, *Alnus nitida* Endl., is from 1 to 1·3 inches in length. The criterion of size may serve as a trustworthy diagnostic feature in living cones but much reliance cannot be placed on it in the study of fossils, especially when one is not sure whether the latter are complete specimens or not; therefore, the author hesitates to specifically determine these specimens.

3. *Bark Fragments*
Betula sp. *cf. B. utilis*
(Plate XXI, Figs. 18, 19)

Two specimens of thin sheets of bark which are identified as belonging to *Betula* may be described as follows:—

Plate XXI, Figs. 18, 19 are natural size photographs of counterparts of a piece of bark from the outer covering of a birch stem. The fossil bark is somewhat greyish-brown in colour, which could not be brought out in the photographs. The specimens do not show any cellular structure on their surface.

Number of specimens.—Four only.

Occurrence.—Laredura, at 6,000 ft., Pir Panjal Range, Kashmir.

Collector.—R. R. Stewart, 1935, G. S. Puri, 1941.

Registered Nos. of figured specimens.—Pl. XXI, Fig. 18=L 245, Fig. 19 = L 246.

The fossils have a fairly close resemblance to bark of *Betula utilis* though it is difficult to prove their identity with this species.

MODERN DISTRIBUTION OF THE BETULACEÆ

The family Betulaceæ is represented in both hemispheres by more than 100 living species belonging to 6 genera. These occur in the north temperate regions of Europe, Asia and America; also in the mountains of tropical countries. In India, we have only four species, which are distributed in two genera; these are exclusively forest trees occurring at the present time throughout the Himalayas.

MODERN DISTRIBUTION OF *Alnus*

Alnus.—The genus includes about 17 modern species of trees and shrubs chiefly represented in the north temperate zones; two species, which have been discovered in the Karewas in a fossil state, extend into the Himalayas and further India; some species occur in Mexico, Europe, within Arctic circle, North Africa, and West and North Asia.

In India we have two species, both of which are hill types occurring chiefly in the Western Himalayas though they have been recorded also from Assam and Burma.

MODERN DISTRIBUTION OF THE FOSSIL SPECIES OF *Alnus*

Alnus nepalensis Don. grows throughout the temperate Himalayas from the Ravi eastwards, usually at an altitude of 3,000–9,000 ft., but

occasionally it occurs at lower altitudes also. It is rather common between Chamba and the Mishmi Hills in Assam; and occurs also in Upper Assam and the Khasi Hills. It extends further east to Ava, on the Khaken Hills and in Yunan.

It has a gregarious growth along streams and moist shady ravines in the Western Himalayas; in the Eastern Himalayas and the hills of Burma it is mostly found on landslips or other exposed soils, but also occurs along moist ravines. In the Punjab Himalayas it is usually met with as a forest tree and rarely occurs along streams or shady ravines.

In the hills around Darjeeling *Alnus nepalensis* grows with *Bucklandia populnea*; both of which are able to colonise newly exposed soil and landslips with remarkable rapidity.

The available data from the authentic sheets of Herbaria seem to show that the species is probably absent from Hazara, the Murree Hills and Kashmir; however, it has been recorded from Bashahr, Kulu, Chamba, Naini-Tal, Garhwal, Nepal, etc.

In the "alder wood forests of Bengal" Champion (1936, p. 260) records it to be growing with *Trema orientalis*. In Kumaon and Garhwal it is associated with *Populus ciliata*, which has been found in a fossil state also at the same locality that has yielded leaves of *Alnus nepalensis*.

Alnus nitida Endl.—"The West Himalayan alder" grows at the present time in the Western Temperate Himalayas from Kashmir to Kunawar at an altitude of 5,000–9,000 ft. It occurs usually at 6,000 ft. in the regions lying westwards from the Jumna Valley. It is always met with along streams, rivers and moist ravines. In the Punjab Himalayas where it appears to be fairly common, it is reported from the Swat Valley, Chitral, Hazara and Kashmir. The Kashmir distribution of the species, with which we are specially concerned here, is rather interesting. It occurs in the Chineni forests in the Jammu Province, Kishtwar, Marwa Dachhan, Muzaffarabad, and Udhampur, which are situated on the outer ranges. The Kashmir Ramban Valley, the northern slopes of the Pir Panjal Range and the southern slopes of the Main Himalayas do not allow the growth of this species on account of changed climatic conditions since the Pleistocene times.

The species extends eastwards to the Parbatti Valley, Bashahr, Simla Hills, Kunawar, and Tehri-Garhwal. It is also common along streams and moist ravines in the dry valleys of the Inner Himalayan ranges. It generally grows in moist ravines in the forests of *Pinus longifolia*.

In the "moist temperate forest", it forms pure patches and occurs along streams in the Beas Valley, Kulu, gregariously with *Cratægus crenulata* (Champion, *loc. cit.*, p. 260).

Modern Distribution of *Betula*

Betula.—The genus includes about 40 modern species of trees or shrubs which are chiefly distributed in the north temperate zone; several species extend into the regions of very high latitudes and one or two are found even beyond the Arctic circle. The species extend as far south as the Himalayas and Southern China in the Eastern Hemisphere and in the New World they have been found in the most southern parts of the United States. Several European species occur in the alpine regions in North-Western and Western Europe, North Asia and North America. *B. nana*, a common British species occurs in Greenland.

Modern Distribution of the Fossil Species of *Betula*

Of the three Indian species, which are hill types, two have been found in a fossil state from the Karewas.

Betula utilis Don. is the high level birch of the Inner Himalayan ranges, which commonly occurs from Bhutan westwards at an altitude of 10,000–14,000 ft.; in the Kishenganga Valley in Kashmir it descends as low as 6,000–7,000 ft. It also extends to Western Tibet and China.

It reaches the uppermost limit of tree growth in the Himalayas and forms pure forests or grows in association with *Rhododendron campanulatum, R. anthopogon*, or sometimes with the conifers, namely, *Abies Webbiana, Juniperus, Larix* and *Pinus excelsa.* In Hazara besides *Pinus excelsa, Abies Webbiana*, and *Picea Morinda* it is associated with *Quercus semecarpifolia, Pyrus lanata, P. foliolosa*, and one or two species of *Salix.* Other genera, which are usually associated with *Betula utilis*, are *Ribes, Berberis, Lonicera, Polygonum, Sorbus*, etc., etc.

In the "alpine fir-birch forests of North Garhwal" it grows according to Champion (*loc. cit.*, p. 270) with *Abies Webbiana, Q. semecarpifolia, Pyrus foliolosa, Rhododendron campanulatum, Cotoneaster acuminata, Rosa sericea, Ribes glaciale, Rubus niveus, Lonicera* spp. and in the forests of Gompatung Chu in the Sikkim Himalayas it grows at an altitude of 12,500 ft. with *Abies densa, Juniperus pseudosabina, Rhododendron Wightii, Pyrus aucuparia, Salix Wallichiana, Juniperus recurva, Spiræa* sp., etc. (Champion, *loc. cit.*, p. 271).

It also occurs in the "birch-*Rhododendron* forest"; in the Sunderdhunga Valley (Western Kumaon) at an altitude of 12,500 ft.; it is associated with

Rhododendron campanulatum, Pyrus foliolosa, Viburnum nervosum, Rhododendron lepidotum, Lonicera parvifolia, Berberis sp. (Champion, *loc. cit.*, p. 272).

In Kashmir, it occurs in the Jhelum Valley, Gurez, Kamraj, Keran, Kishtwar, Marwa Dachhan, Muzaffarabad, Ramban, and the Sindh Valley.

On the northern slopes of the Pir Panjal, Kashmir, it forms a well marked zone at an altitude of 10,500–12,000 ft. and occurs in association with *Rhododendron campanulatum, Salix denticulata, Syringa Emodi, Pyrus foliolosa, Lonicera* sp., etc.

Betula alnoides Buch-Ham. occurs in the Himalayas from the Sutlej eastwards, chiefly in temperate and sub-tropical tracts at an altitude of 5,000–10,000 ft. also in the Khasi Hills, Manipur; and hills of Burma. At the higher altitudes it is generally mixed with oaks, and other broad-leaved species and conifers. In the Punjab Himalayas from Ravi eastwards it occurs in moist ravines.

It may be interesting to point out that this species does not grow in Hazara, the Murree Hills or Kashmir.

It is recorded from the Parbatti Valley, Kulu, the Bashahr State, the Simla Hills, Mussoorie, Naini-Tal and Garhwal.

Past Distribution of the Betulaceæ

Several species of the Betulaceæ have been described from leaf-impressions from Europe and America from the rocks varying in age from the Cretaceous to the Pleistocene. Some of the species have been based upon imperfect specimens, and identifications of quite a few species are unsatisfactory. Several workers have urged for a revision of Tertiary floras of America and Europe and in the absence of such revised treatises it is difficult to compile trustworthy data on the past distribution of the family. Leaves of *Alnus* and *Betula* have been recognised from Tertiary rocks of Europe and America and the presence of the family during this period cannot be denied but the author thinks it advisable to refrain from making list of the species for inclusion in this paper.

Summary

1. The present paper describes some fossil remains of the Betulaceæ from collections made by Middlemiss, Stewart and the author from the Lower Karewa Deposits of Kashmir, exposed along the Pir Panjal side of the valley at Liddarmarg, Ningal Nullah and Laredura. The material includes fossil leaves belonging to *Betula utilis, Betula alnoides, Betula* sp. A (which does

not compare with any modern species of the Himalayas), *Alnus nepalensis*, *Alnus nitida* and a few female cones of *Betula* sp. and *Alnus* sp. (which could not be determined specifically on account of the fragmentary nature of the specimens); a number of impressions, which favourably compare with thin sheets from the outer bark of *Betula utilis* are also present.

2. Of the four well-determined species, only one (*Betula utilis*) occurs in the Kashmir Valley at the present time; the other three species, though still occurring in the neighbouring regions, are absent from the Kashmir Valley proper, the northern slopes of the Pir Panjal Range and the southern slopes of the Main Himalayas.

3. The past and present distribution of the Betulaceæ is briefly discussed.

4. A comparison of the past and present distribution of the Betulaceæ does not alter the general conclusions, which were derived by the author from the general distribution of the Karewa flora as a whole.

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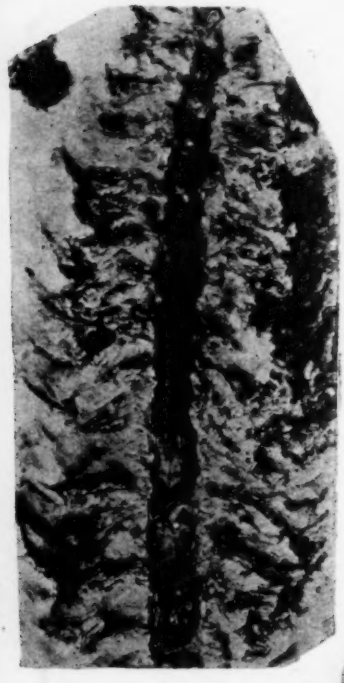

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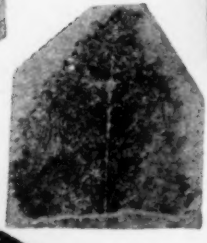

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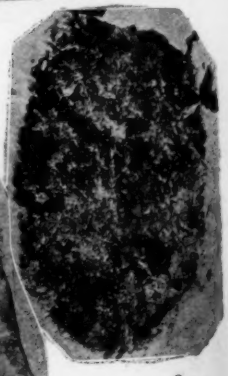

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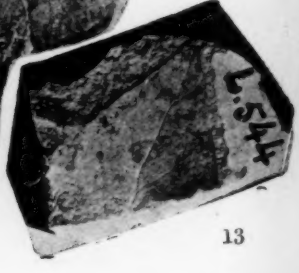

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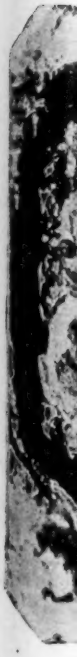

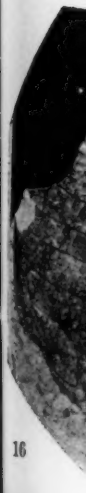

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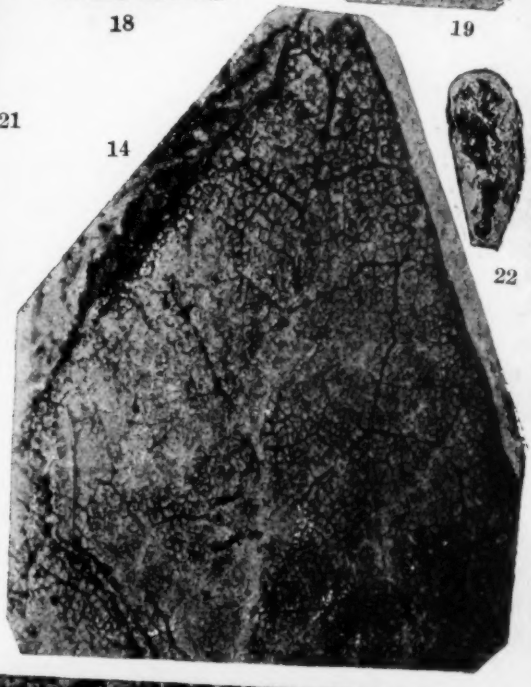

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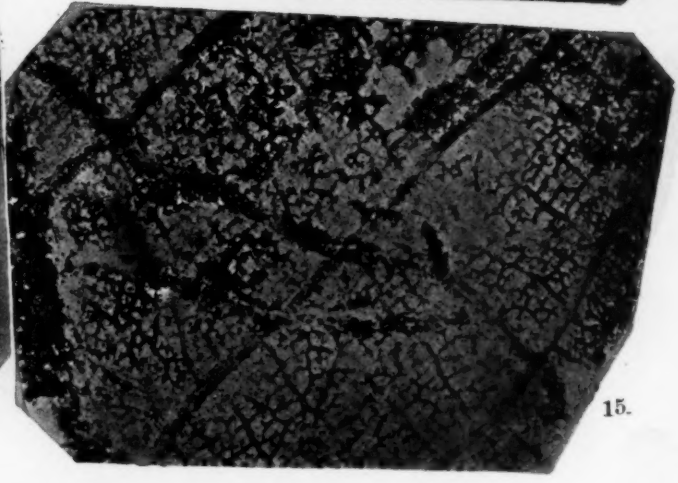

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Explanation of Plates

The photographs in Plates XIX–XXI are from untouched negatives. The figured specimens excepting one (Fig. 16), which is the property of the Geological Survey of India, are presented to Professor B. Sahni.

Plate XIX

Betula utilis D. Don.

Fig. 1. Fossil leaf fragment. R. R. Stewart collection, L 500, Laredura. Nat. size.
Fig. 2. Modern leaf for comparison with Fig. 1. Nat. size.

Betula alnoides Buch.-Ham.

Fig. 3. Leaf (impression of the upper surface). G. S. Puri collection, L 804/3, Laredura. Nat. size.
Fig. 4. A part of the leaf (Fig. 3) enlarged to show the nature of laterals; tertiary and finer reticulation. ×*Ca.* 5.

Plate XX.

Betula sp. A.

Fig. 6. Leaf impression. G. S. Puri collection, L 774/4, Laredura. Nat. size.

Betula utilis D. Don.

Fig. 7. Leaf fragment. R. R. Stewart collection, N 7, Ningal Nullah. Nat. size.
Fig. 8. A part of the leaf (Fig. 7) enlarged to show tertiary and finer reticulations. ×*Ca.* 5.

Betula sp.

Fig. 9. Female cone showing the structure in a longitudinal plane. R. R. Stewart collection, L 25, Laredura. Nat. size.
Fig. 10. The cone in Fig. 9 enlarged to show the scales. ×*Ca.* 5.

Alnus nepalensis D. Don.

Fig. 11. Leaf (impression of the lower surface). R. R. Stewart collection, N 220, Ningal Nullah. Nat. size.

Alnus nitida Endl.

Fig. 12. Leaf (impression of the lower surface). G. S. Puri collection, L 543, Laredura. Nat. size.
Fig. 13. Counterpart of the tip of the leaf (Fig. 12). L 544. ×*Ca.* Nat. size.

Plate XXI

Alnus nitida Endl.

Fig. 14. A part of the leaf (Pl. XIX, Fig. 12) enlarged to show loops of the laterals; tertiary and finer reticulations. ×*Ca.* 5.

Alnus nepalensis D. Don.

Fig. 15. A part of the leaf (Pl. XIX, Fig. 11) enlarged to show tertiary and finer reticulations. ×*Ca.* 5.
Fig. 16. Leaf (impression of the lower surface). Middlemiss collection, K 14/948 (i), Liddarmarg. Nat. size.

Betula sp. A

Fig. 17. Leaf impression. R. R. Stewart collection, L 200, Laredura. Nat. size.

Betula sp. cf. *B. utilis*

Fig. 18. Bark (impression). R. R. Stewart collection, L 245, Laredura. Nat. size.
Fig. 19. Counterpart of Fig. 18. L 246. ×Nat. size.

Alnus sp.

Fig. 20. Female cone. R. R. Stewart collection, N 230, Ningal Nullah. Nat. size.
Fig. 21. Cone (Fig. 21) enlarged to show nature of scales. ×*Ca.* 5.

INCIDENCE OF SOME NEMATODES OF DOMESTIC RUMINANTS IN THE PUNJAB AND UNITED PROVINCES, WITH A NOTE ON THE MORPHOLOGY OF *TRICHURIS GLOBULOSA* (V. LINSTOW)

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Received August 31, 1945

(Communicated by Dr. G. D. Bhalerao, F.A.SC.)

This paper deals with the incidence of trichurids and some trichostrongylids, in the Punjab and the United Provinces (plains and Kumaon hills). The little that is known of the incidence of trichostrongylids in India is supplemented and notable differences in the nematode fauna of the three regions have been noted; some of these parasites were quite common at one place and totally absent from the other. New host records added, are the occurrence of *Trichostrongylus probolurus* in goats, *T. colubriformis* in cattle, *T. axei* and *Ostertagia osteragi* in goats, *O.* (*Camelostrongylus*) *mentulata* in Chinkara deer (*Gazella benneti*), *Trichuris globulosa* in sheep and goats, and *Cooperia punctata* and *C. pectinata* in sheep.

The work was carried out from April to November 1940 at Lahore when, on the average, four goats per week were examined for helminths. Thirty sheep and goats and ten cattle were examined at Mukteswar from the beginning of August to the middle of November 1942 and more than fifty sheep and goats were examined at Izatnagar at different periods of the years 1943 and 1944.

Trichostrongylus probolurus was recorded by Nagaty (1932) from a single male specimen collected from a sheep in the Punjab. This parasite is fairly common at Lahore and infests, in small numbers (about 2 dozen specimens collected at a time), about 50 per cent. of sheep and goats and is usually found associated with *T. colubriformis*. At Izatnagar, I examined sheep and goats for trichostrongylids all the year round but did not find *T. probolurus*. On the other hand, *T. colubriformis* infested about 90 per cent. of animals (cattle, sheep and goats) both at Izatnagar and Lahore. This parasite was first recorded by Giles (1892) and later by Lane (1913).

It is considered by some to be rare in India. *T. axei* was never seen at Lahore but at Izatnagar it was quite common and associated with *T. colubriformis* in small numbers. Its incidence at Mukteswar was similar to that of *T. probolurus* at Lahore.

Ostertagia (*Camelostrongylus*) *mentulata* is the commonest species of the genus in sheep and goats at Lahore and infests about 50 per cent. of them. It was also collected from a Chinkara deer (*Gazella benneti*) which died at the Zoological Gardens, Lahore. The other three species mentioned below appear to be confined to the hills.

O. ostertagi was collected twice from hill-goats at Lahore but was never seen in plains-goats. It is already known from cattle at Mukteswar. A single female with a striated tail was found mixed with *O. ostertagi* and seems to represent a different species.

O. circumcineta was recorded by Bhalerao (1932) from goats at Mukteswar and I have observed it in sheep in the same locality.

Cooperia punctata and *Cooperia pectinata* were very often collected from hill bulls at Mukteswar and were, sometimes, the only nematode parasites in the intestine. The first three feet of the small intestine harboured about 20 worms per inch. The large number and the symptoms exhibited by the hosts, suggested their being the cause of death. A characteristic symptom was the weakness of legs, the animal eating and drinking in a recumbent position. Microscopic examination of fæces of newly arrived animals always revealed their presence. These parasites, first recorded by Rao (1940) from cattle at Madras, are the commonest trichostrongylid parasites of cattle in the Kumaon hills and Bareilly district. Both the species occurred in two collections from sheep at Mukteswar.

Trichuris globulosa.—Fifty sheep and goats were examined for this parasite at Lahore and some in the slaughter-houses at Multan, Sialkot and Delhi and in the post-mortem room at Mukteswar. Dr. H. D. Srivastava kindly allowed me to examine some collections brought by him from Peshawar and Karachi. Of the 120 animals examined, in all, 112 were found to be harbour whipworms. Some fifty cattle were also examined at the various localities named above but only two were found to harbour trichurids. It is interesting to note that they were neither *T. globulosa* nor *T. ovis*. *T. globulosa* was invariably present in all the collections from sheep and goats and it is interesting that although *T. ovis* has been considered by many workers in this country to be a very common parasite, yet it was never encountered by the writer.

A study of literature on the whipworms of ruminants has revealed some confusion as to the specific identity of the species mentioned. Sprehn (1927) pointed out that *T. globulosa* and *T. ovis* had been badly mixed up and suggested that *T. alcocki* (V. Linstow) was a synonym of *T. globulosa.* Baylis (1932) found that some material previously determined as *T. ovis* in the British museum collection was really *T. globulosa* and pointed out that this species was, probably, at least, as common as *T. ovis* in domestic ruminants in Uganda and Zululand and was, perhaps, widely distributed in East and South Africa. Mönning (1933) and Ortlepp (1937) examined all the available material at Onderstepoort and found that all the *Trichuris* material from sheep, goats, cattle and wild antelopes was *T. globulosa.* Baylis (1939) considers *T. cameli* to be, probably, identical with either *T. globulosa* or *T. ovis.* According to Gaiger (1910) Leese found *T. cameli* in a dromedary in India. The writer examined Leese's collection of *T. cameli* consisting of two males and several females in a very bad state of preservation at the Punjab Veterinary College. The males were not recognisable but the females were all identical with *T. globulosa.*

This confusion in the group is attributed to morphological variations in the individuals of a species. *T. globulosa* and *T. ovis* being relatively closely allied, have probably presented similar difficulties in their specific differentiation. The writer has given below the specific points of *T. globulosa* and has described its internal genitalia. Most individuals in the various collections were strikingly similar but all the individuals from a goat at Mukteswar presented constant variations in the shape of vagina, length of spines on the posterior part of sheath, in their absence from the posterior part of the bulb and smaller size of vas deferens and ejaculatory duct. A short account of these atypical specimens is given later. Ortlepp (1937) states that he has seen individuals of *T. globulosa* having melon-shaped swellings of sheaths. This character is considered by Baylis (1932) and Gebauer (1932) to be specific for *T. ovis.* The individuals in the writer's collection usually had sheaths with semi-everted distal swellings and the fully everted sheaths only had globular swellings. Spines on the sheath were always closely set, sharply pointed and backwardly directed, measuring 0·02–0·025 mm. on the swelling and 0·01–0·012 mm. on the proximal part. The spicules were 4·4–6·5 mm. long with 'flares' on the anterior part varying from 0·041–0·085 mm. in thickness. In certain individuals they were uniformly thick. Thickness in one case was 0·05 mm. at the 'flares' at the anterior end; 0·05 mm. at the middle and 0·051 mm. at the posterior end before thinning. Thinning of the margins of the spicule at the point of thickness at the posterior end is a usual feature and

gives a false impression of thickness. The most important constant feature observed is the ending of the spicule in a sharp tip (Fig. 1, *b*). In two collections all the worms had weakly cuticularized spicules—a character given as specific for *T. antidorchi* by Ortlepp (1937). The writer could generally rely on the shape of the posterior end of the body with a characteristic dip (Fig. 1, *a*) proceeded by the rounded lobe for identification of *T. globulosa.* The depth of the 'dip' varies according to the contraction of the part preceding it. Vas deferens measures 9·4–11·5 mm. long while the ejaculatory duct is 7·5–9·9 mm. Cloaca is 2·9–3·9 mm. long with the spicular tube joining it 1·7–2·225 mm. from the posterior end. A small muscular constriction is present at the junction of vas deferens with the ejaculatory duct. Breadth of vas deferens, just before the junction, is 0·35–0·465 mm. and the breadth of the ejaculatory duct near its commencement is 0·4–0·56 mm. The characteristic appearance of vagina described by Baylis (1932) was constant. In cases of engorgement with eggs the angular beds were rounded.

Description of the 'atypical' collection is as follows:—Male: Length of anterior part 30–32 mm.; length of posterior part in the middle 0·49–0·52 mm. and breadth before ending 0·28–0·3 mm. The spicules were 4·4–4·8 mm. long and 0·039–0·042 mm. broad in the middle. Sheaths in all the specimens were sub-globular with a part of the distal swelling devoid of spines (Fig. 2, *a*). Spines on the distal swelling measured 0·029–0·03 mm. and on the proximal part 0·009–0·01 mm. The testis starts just behind the cloaca; it is lightly convoluted in front of the ejaculatory duct and is markedly convoluted in front of the vas deferens. Length of the ejaculatory duct is 5·5–6·1 mm. and that of vas deferens 6·6–6·9 mm. Breadth of ejaculatory duct just near its beginning is 0·29–0·34 mm. while that of vas deferens before ending is 0·23–0·25 mm. The cloaca measures 1·85–2·2 mm. in length and the spicular tube joins it 0·01–0·011 mm. from the posterior end. Female: Length of the anterior part was 29–32 mm. and that of the posterior 13–15 mm.; maximum breadth of the posterior part was 0·89–0·92 mm. The vagina has angular beds but a remarkable feature of all the females is the presence of secondary muscular buddings alternating with large ones (Fig. 2, *b*). Eggs including opercula, measured 0·049–0·055 mm. long and 0·029–0·03 mm. broad.

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FIG. 1. *T. globulosa*, sheath and spicule

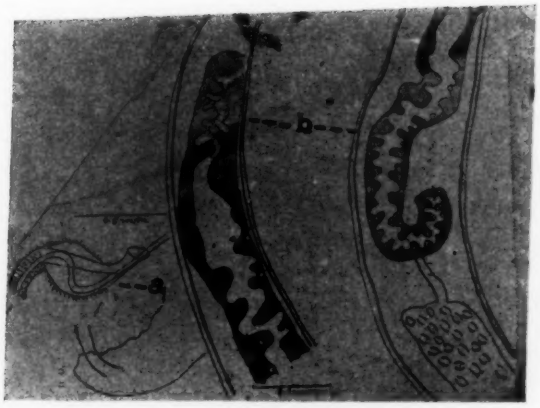

FIG. 2. *T. globulosa*, sheath and vagina

SOME FOSSIL LEAVES AND FRUITS OF THE ACERACEÆ FROM THE KAREWA DEPOSITS OF KASHMIR, WITH REMARKS ON THE PAST AND PRESENT MAPLE FORESTS OF THE KASHMIR VALLEY

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Received July 5, 1945

(Communicated by Professor Birbal Sahni, F.A.SC., F.R.S.)

(With Six Plates)

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Introduction

The material described in this paper comprises a score of fossil leaves and a few fruits of the genus *Acer*, which come from the Lower Karewa deposits of Kashmir. Most of the specimens are derived from the author's collections of 1939 and 1940 and some from Stewart's collections of 1935 and 1936

from the beds exposed along the Pir Panjal side of the valley at Laredura and Ningal Nullah; however, there are four leaf-fragments of *Acer oblongum*, two samaras and an incomplete leaf of *Acer pentapomicum*, which are described from collections made from Liddarmarg (from two different spots) by Middlemiss (1911, pp. 121–22) in 1910 and de Terra in 1932.

I am highly indebted to Prof. B. Sahni, F.R.S., for guidance and encouragement during the preparation of this paper. I am equally grateful to my old teacher Dr. R. R. Stewart, for helping me in identification of the fossil leaves, and for innumerable facilities extended to me during my stay in the Gordon College for the period of two years (1937–1939). It is my pleasant duty to thank the Director, Geological Survey of India, for the loan of Middlemiss's collection and for the photographs of *A. oblongum* reproduced in Plate XXIII. My grateful thanks are also due to the Vice-Chancellor, University of the Punjab and Principal Jodh Singh of the Khalsa College, Amritsar, for a research scholarship from the University and the authorities of the Lucknow University for the award of a Research Fellowship for my work on the Karewa floras at Lucknow. I am glad to acknowledge the assistance my wife has given me in correcting the proofs.

Plant-bearing Localities

A detailed description of the localities is given elsewhere (Puri, 1944) but a brief account of these may be given here as follows:—

Locality L.—Laredura, near Dangarpur, seven miles from Baramulla towards Gulmarg, height about 6,000 ft., above sea-level.

Locality N.—Ningal Nullah, about five miles south of Dangarpur village in a grassy meadow about a furlong from the bridge over the Ningal Nullah stream and near Botapathri village at an altitude of 9,000 ft.

Locality 3 *L.*—" Liddarmarg, in the stream bed 500 ft., west of the shepherds' huts, the beds dip 6 degrees north-east and are at 10,600 ft. "

Locality K 14/948.—" Liddarmarg, in two stream beds near the present Gujar (herdsmen) encampment, the dip of the bed is of the usual slightly inclined character, the altitude is 10,600 ft. above the sea-level."

Systematic List of the Species

Acer oblongum Wall.

Acer pentapomicum J. L. Stewart.

Acer villosum Wall.

Acer pictum Thunb.

Acer sp. A

Acer sp. B

Acer sp. C

Acer sp. D

Acer Cæsium Wall.

Acer sp.

DISTRIBUTION OF THE FOSSIL SPECIES IN THE KAREWAS

	Laredura alt. 6,000 ft.	Ningal Nullah alt. 9,000 ft.	Liddarmarg alt. 10,600 ft.
Acer oblongum ..			×
Acer pentapomicum ..		×	×
Acer villosum ..	×	×	
Acer pictum ..		×	
Acer sp. A ..	×		
Acer sp. B ..		×	
Acer sp. C ..	×		
Acer Cæsium ..		×	
Acer sp. D ..	×		
Acer sp. ..	×		
Acer sp. ..			×

DESCRIPTION

The family Aceraceæ is represented in the Karewa floras by five fully determined and five incompletely identified species; out of the latter five, four are based on leaf fragments, which in addition to being distinct from one another, do not seem to match any modern species of *Acer* growing at the present time in the Himalayas. The fifth species is based on a few fragments of fruits, which are difficult to determine specifically on account of the lack of knowledge regarding the fruits of modern species.

Leaves of *Acer*, whether modern or fossil, possess a definitely characteristic shape, which together with venation and nature of the margin of the lobes (if the leaf is lobed) serve as diagnostic features for different species.

Most of the leaves are fragmentary but they are well preserved as regards details of venation. One leaf fragment from Stewart's collection from Laredura was heavily coated in the field with rubber solution, which has greatly obscured the tertiary and finer reticulation, with the result that they have not been brought satisfactorily in the natural size photograph of the specimen.

KEY TO THE SPECIES

Leaves.—

I. Leaves undivided .. .. *A. oblongum*

II. Leaves palmatilobed.—

(i) Three-lobed:

(*a*) Margin entire.—Large, lobes acute at apex, laterals conspicuous, parallel and form two or more series of distinct loops below the margin .. .. *Acer* sp. B

Small, lobes acuminate at apex, laterals not so conspicuous, upper somewhat parallel, do not form loops below the margin .. .. .. *Acer* sp. A

(*b*) Margin definitely crenate, laterals fairly conspicuous, do not form clear loops below the margin *Acer villosum*

(*c*) Margin doubtfully toothed, laterals quite conspicuous, bifurcate below the margin and form loops .. .. *Acer pentapomicum*

(ii) Five-lobed: Large, laterals more or less obsolete *Acer* sp. D

(iii) Lobes either 3 or 5 (?): Large, laterals conspicuous and arcuate .. .. *Acer* sp. C

(iv) Five- or seven-lobed: Large, laterals somewhat conspicuous, soon break up in the lamina to form meshes .. .. *Acer pictum*

Fruits.—

I. Large.—Nut not preserved, reticulations of the wing large and clear .. .. *Acer Cæsium*

II. Small.—Nut preserved, reticulations fine and small .. *Acer* sp.

1. *Leaves*

Acer oblongum Wall.

(Plate XXIII, Figs. 1–4 and Pl. XXIV, Fig. 5)

The specimens referred to this species are four leaf-fragments, which are well preserved impressions as regards details of venation. Pl. XXIII, Fig. 2 is a natural size photograph of a complete leaf; there are two more leaf fragments, which are almost complete; the fourth specimen (Pl. XXIII, Fig. 3) is entirely broken on one side along its breadth. The complete leaf is broadly ovate in outline with a broad rounded base and acute apex. The margins are entire.

The venation is pinnate-looped with a tri-nerved base. A strong midrib starts from the base and runs straight in the lamina, gradually thinning out towards the apex. In Pl. XXIII, Fig. 2 the two basal secondaries, which are as strong as the midrib, arise very close to the base on its either side in opposite fashion and run straight towards the margins; the basal pair and the midrib form a tri-nerved base, which gives a pseudopalmate appearance to the venation. The midrib gives off, above the base, 8–9 secondaries on either side at slightly obtuse angles. The laterals, which are well preserved and about half as thick as the midrib, shoot out mostly in an alternate manner but there are some which are sub-opposite. The basal pair of secondaries gives off a number of small branches on its lower side (Pl. XXIII, Fig. 2). At some places there arise between the two strong laterals one or two weak secondaries, which are very thin and do not reach the margins but break up into large meshes in the lamina. The midrib and the secondaries have left shallow grooves in some specimens (Pl. XXIII, Fig. 2 and Pl. XXIV, Fig. 5), which seem to be impressions from the lower surface of the leaf; the other two leaf-fragments are probably impressions from the upper surfaces. The laterals bifurcate into two branches, which form loops below the margins (Pl. XXIII, Fig. 4). Tertiary ribs are numerous; they may arise from the midrib, the laterals, or the branches of the laterals, and anastomose with one another to form large meshes of various shapes and sizes in the area enclosed by the two secondaries. These tertiary meshes are clearly seen in the photograph which represents a small part of a fragment enlarged to five diameters. There is a well preserved finer reticulation, which consists of a network of smaller meshes occurring in a large tertiary mesh.

Our fossil leaves are identical in all respects with *Acer oblongum*, a simple leaved Himalayan maple, which is also found in plains.

No. of specimens.—Ten only.

Occurrence.—Liddarmarg, at 10,600 ft., Pir Panjal, Kashmir.

Collections.—C. S. Middlemiss, 1910, and H. de Terra, 1932.

Registered Nos. of figured specimens.—Pl. XXIII, Fig. 1 = K14/948 *a*; Pl. XXIII, Fig. 2 = K14/948 *a* 23; Pl. XXIII, Figs. 3, 4 = K14/948 *a* 13″; Pl. XXIV, Fig. 5 = K14/948 *a* 9.

Acer pentapomicum J. L. Stewart

(Plate XXIV, Figs. 6–8)

This species is based on two leaf-fragments, one of which (Pl. XXIV, Fig. 8) is evidently a three-lobed leaf, but the other specimen (Pl. XXIV,

Fig. 6) may have had probably a five-lobed lamina. The former specimen comes from Liddarmarg and the latter was collected from Ningal Nullah. The Liddarmarg fragment, which measures 2·1 inches long by 1·5 inches in the broadest part, must have been broader than long. Leaf lamina is palmatifid with two completely preserved lobes, which are more or less ovate in outline. Margins of the lobes are entire; apices are acute, and the base of the leaf lamina seems to have been slightly rounded.

The Ningal Nullah specimen is palmati-partite; there is a wide sinus between the two lobes; and the fragment is broader than long and measures 1·3 inches long by 1·8 inches in the broadest part. The lobes are ovate in shape with acute apices and irregularly coarsely serrate margins; the teeth are blunt.

The venation is palmate-reticulate consisting probably of 3 primary ribs, which arise from the base of the leaf and end in the apices of the lobes. In both the fragments, only two primary ribs are preserved. The primary veins give off a number of secondaries at slightly acute angles in a pinnate manner; the secondaries, which are about half as thick as the main ribs, run straight in the lamina and bifurcate near the margins into two relatively thinner branches, each of which forms a small loop by anastomosing with branches of the superior and inferior laterals (Pl. XXVI, Fig. 7). The loops are ovate in shape and their convexities face the margins. Tertiary ribs arise from the main ribs as well as the secondaries and anastomose variously to form meshes of different shapes and sizes in the area enclosed by the two laterals (Pl. XXIV, Fig. 7). There is a finer reticulation consisting of a network of small polygonal or rectangular meshes, which are occupied in the large meshes of the tertiary reticulations.

Our fossil leaves are identical in all respects with *Acer pentapomicum* J. L. Stewart, a small tree of the inner Himalayan dry valleys.

Number of specimens.—Four only.

Occurrence.—Liddarmarg, at 10,600 ft. and Ningal Nullah, at 9,000 ft. Pir Panjal, Kashmir.

Collectors.—H. de Terra, 1932 and G. S. Puri, 1940.

Registered Nos. of figured specimens.—Pl. XXIV, Figs. 6, 7 = Loc. 1 N6; Pl. XXIV, Fig. 8 = Loc. 3 L 16″.

Acer villosum Wall.

(Plate XXIV, Figs. 9, 10)

The specimen described here is a palmately lobed leaf of which only two lobes are preserved, the third being greatly damaged. The fragment is

broader than long and measures 1·8 inches long by 2·3 inches in the broades part. The lobes are somewhat ovate in shape; the lower lobe is probably smaller than the middle and the sinus between the two lobes is narrow and acute. The margins are irregularly crenate. The lobes have acute apices. Base of the leaf seems to be cordate.

The venation is palmate and reticulate consisting of three primary ribs each of which runs straight in its respective lobe. The primary ribs give off on either side at acute angles a number of secondary veins, which can only be seen in the complete lobe in the photograph (Pl. XXIV, Fig. 9). The secondaries are obscured in the rest of the leaf due to the application of a thick coat of rubber solution. The laterals, which are about half as thick as the primary ribs, diverge in an alternate manner. They give off a number of smaller branches on both sides and the latter being very thin are often indistinguishable from the tertiary ribs, which form large rectangular or polygonal meshes; the meshes can be clearly seen in a part of the leaf, which is enlarged to five diameters in the photograph (Pl. XXIV, Fig. 10). There is a finer reticulation, which consists of a fine, well preserved network of smaller meshes that are occupied in the tertiary meshes.

The fossil leaf is identical in all respects with *Acer villosum* Wall., a large tree of the Western Himalayas.

Number of specimens.—Six only.

Occurrence.—Laredura, at 6,000 ft. and Ningal Nullah, at 9,000 ft., Pir Panjal, Kashmir.

Collectors.—R. R. Stewart, 1936 and G. S. Puri, 1940.

Registered No. of figured specimen.—L 193.

Acer pictum Thunb.

(Plate XXV, Fig. 11)

Plate XXV, Fig. 11 is a natural size photograph of a leaf-fragment, which measures 2·9 inches long by 3·5 inches in the broadest part. The leaf-lamina is palmately lobed; the number of lobes, on account of the fragmentary nature of the specimen, cannot be definitely ascertained, but from the number of the primary veins it appears that the leaf was probably 5 or 7 lobed. Base of the leaf seems to be cordate.

The venation is palmate-reticulate consisting of 7 main ribs, which are fairly stout and diverge from the base of the leaf towards the lobes in the apex of which they end. The photograph shows one badly preserved lobe in which the main rib is seen tapering gradually towards the apex. The

laterals make slightly deep grooves in the fossil, which seems to be an impression from the lower surface of the leaf.

The primary ribs give off a number of small secondary veins, which are comparatively very thin and feeble. The secondaries do not run straight but fluctuate in the lamina following a zigzag and wavy course. They diverge from the main ribs at different angles, and on account of their extremely thin nature soon get lost in the tertiary reticulation, which is also composed of ribs of almost the same thickness. Meshes of the tertiary reticulation are more or less rectangular or roughly pentangular in shape. There is a finer reticulation, which is composed of very small rectangular or polygonal meshes occupied in the large tertiary reticulations.

Our fossil fragment is identical in all respects with *Acer pictum* Thunb., a handsome medium-sized tree of the Himalayas.

Number of specimens.—Six only.

Occurrence.—Ningal Nullah, at 9,000 ft., Pir Panjal, Kashmir.

Collector.—G. S. Puri, 1940.

Registered No. of figured specimen.—Loc. 1 N7.

Acer sp. A

(Plate XXV, Figs. 16, 17)

Plate XXV, Fig. 16 is a natural size photograph of a three-lobed leaf, which measures 1·9 inches long by 2·3 inches in the broadest part. One of the basal lobe is partly missing in the leaf; the two others also are slightly broken at the tips. The leaf is slightly broader than long with an ovate outline and a cordate base. Each lobe which is also ovate in outline is broader at the base and abruptly tapers into an acuminate apex. The sinus between the two lobes is wide and obtuse and it extends to about one-third of the way in the lamina; the margins are entire.

The venation is palmate-reticulate. It consists of three main ribs, which run straight and end in the apices of the lobes. The primary ribs give off five to six pairs of lateral veins, which arise at acute angles in a pinnate manner. The laterals are almost half as thick as the primaries. The secondary ribs which arise in the basal lobe on the outer side from the primaries, make a sharp curve under the margins and anastomose with the superior secondaries to form a series of simple loops (Pl. XXV, Fig. 17). The secondary ribs that arise from the inner side of the basal primary rib anastomose with the secondaries that arise from the primary rib of the central lobe. The tertiary reticulation is in the form of

large meshes, which are of different sizes and shapes. There is a well preserved finer reticulation, which is clearly seen in the photograph (Pl. XXV, Fig. 17).

The fossil nicely resembles modern leaves of the genus *Acer*, but it does not match any living species that now grows in Kashmir or anywhere in the north and north-west Himalayas and it may belong to a species, which has become extinct from this part of India. Comparisons with foreign species in the Herbarium of the Forest Research Institute, Dehra Dun, also failed to attain its specific determination.

Number of specimens.—One only.

Occurrence.—Laredura, at 6,500 ft., Pir Panjal, Kashmir.

Collecter.—G. S. Puri, 1940.

Registered No. of figured specimen.—L 670.

Acer sp. B

(Plate XXVI, Figs. 21–23)

This species is based on a three-lobed simple leaf of *Acer*, which may be described as follows. The leaf is broadly ovate in shape and is broader than long. It measures 3·5 inches long by 4·6 inches in the broadest part. Base of the leaf though slightly broken, seems to have been cordate. The lobes are ovate with broad basal regions and narrow tapering apices. The sinus between the lobes is somewhat acute in shape and extends about half way in the lamina. Margins of the lobes are entire. The lateral lobe on the left-hand side in the photograph (Pl. XXVI, Fig. 21) is fractured and the broken fragments are lying jumbled together under and over the leaf.

The venation is palmate-reticulate; it consists of three main primary ribs, each of which diverges from the base and runs in its respective lobe in an almost straight course. Each primary rib gives off 10–15 pairs of secondary veins which are conspicuous and almost half as thick. The secondaries are arranged on a primary rib in a pinnate manner; they shoot out at slightly acute angles and after running straight for about three-fourth of its length curve upwards to form near the margin a series of loops which anastomose to form an infra-marginal vein below the margin (Pl. XXVI, Fig. 22). The tertiary reticulation is composed of large meshes, which are of different shapes. They break up into smaller meshes, which constitute the finer reticulations. Both tertiary and finer reticulations are clearly seen in the photograph (Pl. XXVI, Fig. 23), which represents a part of the leaf enlarged to five diameters.

The fossil does not resemble any living species of *Acer* represented in the present-day flora of Kashmir and the north-west Himalayas. Comparisons with foreign species also failed to determine it specifically.

Number of specimens.—One only.

Occurrence.—Laredura at 6,500 ft., Pir Panjal, Kashmir.

Collector.—G. S. Puri, 1939.

Registered No. of the figured specimen.—L 547.

Acer sp. C

(Plate XXVII, Figs. 24–25)

Although this species shows some resemblance to the fossil leaves referred to *Acer* sp. A and *Acer* sp. B, it seems to be clearly distinct from them and may be described separately. Pl. XXVII, Fig. 24 is a natural size photograph of the leaf-fragment. On account of the fragmentary nature of the specimen it cannot be definitely ascertained whether it is a three-lobed leaf or the number of lobes is five or more. It measures 2·8 inches long by 3·7 inches in the broadest part. The leaf-lamina, which is broader than long, had probably an ovate shape; the base of the leaf and the apices of the lobes are not preserved. The sinus between the lobes is somewhat acute and seems to run fairly deep in the lamina. The margins are entire.

The venation is palmate-reticulate; three main primaries traverse the three incompletely preserved lobes, and give off a number of secondary veins in a pinnate manner. The secondaries arise at slightly obtuse angles and a few pairs in the upper part of the central lobe gradually curve upwards and become arcuate near the margin. Some secondaries, which arise in the lower part of the middle lobe, do not reach the margin but begin to break up and anastomose with inner secondaries of the lateral lobes. These secondaries are relatively thinner than the upper secondaries, which are about half as thick as the primary ribs. A few upper pairs of secondaries form a single series of simple loops, which anastomose with one another to form an inframarginal vein running close to the margins. In between the two strong secondaries there arise one or two weaker secondaries, which soon break up and anastomose to form meshes of the tertiary reticulations. These meshes are large, and are of different shapes. The finer reticulation consists of very small meshes, which are occupied in by the larger tertiary meshes.

The fossil leaf does not resemble any living species of *Acer* growing in India at the present time and it seems to be a new species, which have become extinct from this part of the world. It shows some resemblance to *Acer*

sp. A and *Acer* sp. B, but the differences are fairly well marked out to constitute it a new species. The differences between the three species are brought out in the following table:—

Characters	*Acer* sp. A (Pl. XXV, Figs. 19, 20)	*Acer* sp. B (Pl. XXVI, Figs. 21-23)	*Acer* sp. C (Pl. XXVII, Figs. 24-25)
1. Size ..	Small leaf	A larger leaf about twice or sligtly more than sp. A	It is largest of the three species. The fragment, which is about half of a leaf is more or less of the same size as sp. B
2. Lobes ..	Definitely three-lobed	Definitely three-lobed	May be three-lobed or more
3. Lateral veins	Thin and more or less obsolete. Not arcuate. Outer laterals in the lateral lobes and upper in the central lobe form indistinct simple loops. The laterals in the lower part of the central lobe and those arising from the inner side in the lateral lobes break up and anastomose to form tertiary reticulation	Very well developed and prominent. Slightly arcuate. All laterals form a series of loops, which anastomose to form an infra-marginal vein running parallel to the margins	Well developed and prominent. Very arcuate; only the upper laterals in the central lobe and outer laterals of the outer lobes form a single series of loops which anastomose to form an infra-marginal vein running close to the margin. The laterals in the lower portion of the central lobes are of the same nature as those in species A

Number of specimens.—One.

Occurrence.—Ningal Nullah at 9,000 ft., Pir Panjal, Kashmir.

Collector.—G. S. Puri, 1940.

Registered No. of figured specimen.—Loc. 1 N 8.

Acer sp. D

(Plate XXVIII, Figs. 26–27)

Plate XXVIII, Fig. 26 is a natural size photograph of a leaf-fragment; and a portion of its counterpart is illustrated in Fig. 27 on the same plate. In the counterpart, the margin and apex of one of the lateral lobes is clearly preserved. The palmately lobed leaf, which is probably 5 lobed, is distinctly broader than long and measures 2·6 inches long by 5 inches in the broadest part. Base is cordate; the margins of the lobes are entire. The sinus between the lobes is not clearly seen in the photographs on account of the fractured nature of the leaf. Apex of one lobe, which is completely preserved, is acuminate.

The venation is palmate-reticulate consisting of 5 prominent main ribs, which have left deep grooves in Fig. 27 and stand out in the form of ridges

in Fig. 26. The former specimen is an impression from the lower surface of the leaf, while its counterpart (Fig. 26) represents the upper surface. The main ribs diverge from the base in a palmate fashion and give off in the lamina a few very obscure laterals, which are neither prominent enough to be distinguished from tertiary ribs, nor they seem to reach the margins in the case of the outer ribs. A close meshwork with large meshes, here and there, constitutes what may be called a finer reticulation.

The leaf fragments do not compare any modern species of *Acer* represented in the present-day flora of the Himalayas; therefore, it is described here as a distinct type under *Acer* sp. D.

Number of specimens.—Two (counterparts of a leaf).

Occurrence.—Laredura, at 6,000 ft., Pir Panjal Range, Kashmir.

Collector.—G. S. Puri, 1940.

Registered Nos. of figured specimens.—Pl. XXVIII, Fig. 26 = L 803 *a*; Pl. XXVIII, Fig. 27 = L 803 *b*.

2. *Fruits*

Acer Cæsium Wall. (vel. affinis)

(Plate XXV, Figs. 12, 13)

The specimen described under this species is fragment from a wing of one half of a double samara. The seed portion of the fruit is altogether missing and the wing too is not completely preserved. It measures 1·55 inches long by ·6 inch in the broadest part. The wing is straight and shows a network of strong veins. The veins seem to diverge from the thickened side along its entire length; they arise at slightly obtuse angles and run straight towards the other edge of the wing; most of the veins soon start bifurcating dichotomously and go on repeatedly forking up to the edge. The forked branches anastomose with one another and form a sort of network.

Number of specimens.—One only.

Occurrence.—Laredura, at 6,000 ft., Pir Panjal, Kashmir.

Collector.—R. R. Stewart, 1935.

Registered No. of figured specimen.—L 160.

Our fossil seems to have a close resemblance in size, shape, reticulation, etc., with modern samara of *Acer Cæsium* Wall. (Pl. XXV, Fig. 13).

Acer sp.

(Plate XXV, Figs. 14, 15)

The present species is based on a winged nut of a double samara of *Acer*. Pl. XXV, Figs. 14, 15 are natural size photographs of a samara and its counterpart. Neither of the specimens is well preserved. The entire specimen measures 1·28 inches in length, and consists of the lower seed portion (seen in the photograph as a thick dark diamond-shaped body), which is ·33 inch long by ·25 inch in the middle broadest part. The upper thin membranous part is the wing. From the thick and solid side of the wing there arise a large number of veins, which traverse the body of the wing. These veins are seen in the form of reticulations in the photograph.

As the fossil is only half of a double samara, it is not possible to specifically determine it. No systematic book on the Indian flora gives any description of the samaras; therefore, the identification could not be attained with our present state of knowledge.

Number of specimens.—One and its counterpart.

Occurrence.—Liddarmarg, at 10,600 ft., Pir Panjal, Kashmir.

Collector.—H. de Terra, 1932.

Registered Nos. of figured specimens.—Pl. XXV, Fig. 14 = Loc. 3L 50; Pl. XXV, Fig. 15 = Loc. 3L 51.

Acer sp.

(Plate XXV, Figs. 18, 19, 20)

Plate XXV, Figs. 18, 19, 20 illustrate in natural size three specimens of fruits of *Acer*, which are again very difficult to assign to their species. Two of them (Pl. XXV, Figs. 18, 19) seem to have some resemblance in shape and size and may have belonged to the same species but the third specimen (Pl. XXV, Fig. 20) in its shape seems to be different. All specimens are represented by a nut and a complete wing, in which reticualations are clearly seen. The nut is more or less oblong in Figs. 18 and 19, but in Fig. 20 it seems to be rather four-sided and shows some pitted structure.

As it is not possible to determine any of these samaras specifically, they are described here under *Acer* sp.

No. of specimens.—Three only.

Occurrence.—Laredura, at 6,000 ft., Pir Panjal Range, Kashmir.

Collector.—G. S. Puri, 1939.

Registered Nos. of Figured specimens.—Pl. XXV, Fig. 18 = L 720; Pl. XXV, Fig. 19 = L 721; Pl. XXV, Fig. 20 = L 549.

Modern Distribution of the Aceraceæ

The family Aceraceæ, which includes more than 150 species belonging to two genera *Dipteronia* and *Acer* is, at the present time, confined to the Northern Hemisphere mostly in the north temperate region and in the mountains of tropical countries.

The two species of *Dipteronia* are confined to Western China and the second genus *Acer*, which includes about 150 modern species, occurs in the Northern Hemisphere. The maples are mostly hill species well represented in Europe, North America and Asia. The region lying between the Eastern Himalayas and Central China has the greatest number of species. Out of a total of 85 species reported from China as many as 67 are confined to this country, which is probably the richest in the world for Acerous plants. Quite a large number of species occur in Japan also. In Europe, the Balkan peninsula and the Western Caucasus are the richest regions for maples, which are distributed throughout the Mediterranean countries. In America, the species of *Acer* are found in the mountains of South Canada, and the region between Oregon and Mexico. It may be interesting to point out that the species of *Acer* found in the Atlantic countries are different from those reported from the Pacific side.

In India there are about 15 species of *Acer*, which are hill types; all of these with the exception of two species (one of which *A. isolobium* is confined to Burma, and the other *A. niveum* occurs both in Assam and Burma), occur in the Himalayas. Some of the Himalayan species, *e.g.*, *A. oblongum* and *A. lævigatum* also occur outside the Himalayas and they are recorded from Assam and Manipur; another Himalayan species *A. Thomsoni* extends as far east as Assam and Burma.

Three species, namely, *A. pentapomicum*, *A. villosum* and *A. Cæsium*, are Western Himalayan while the following six, *A. Hookeri*, *A. sikkimense*, *A. stachyophyllum*, *A. Thomsoni*, *A. Papilio*, and *A. Campbellii*, are distributed in the Eastern Himalayas. There are four species, namely, *A. oblongum*, *A. lævigatum*, *A. caudatum* and *A. pictum*, which are common to both the Western and the Eastern Himalayan regions.

Modern Distribution of the Fossil Species

Our fossil types include three species, which are exclusively Western Himalayan in their present-day distribution and the remaining two species inhabit both the Eastern and Western Himalayas.

(i) *Acer oblongum* Wall., a low-level Himalayan species, which has been discovered in the fossil state at an elevation of 10,600 ft. at Liddarmarg

in the Pir Panjal, is met with at the present time in the outer Himalayan ranges and valleys from the Jhelum to Bhutan, ascending from plains to the altitude of 6,000 ft. It is also recorded from Assam and Manipur. At lower elevations the tree usually occurs in moist ravines and on river banks; it grows along the banks of Kotri river in the United Provinces at 2,000 ft. and is found even at lower altitudes in the Dehra Dun Valley. In the low-lying valleys of Siwalik Hills it is common. In plains it occurs as a cultivated tree. In Kashmir it is found only at Chineni and Mirpur and is also recorded from the Murree Hills, Bashahr, Simla, Mussoorie (to 6,500 ft. alt.), Garhwal, Naini-Tal, Kumaon, etc.

(ii) *Acer pentapomicum* J. L. Stewart, is usually a tree of the Inner Himalayan dry valleys but it also occurs in the Western Himalayas between the Jhelum and the Sutlej Valley at altitudes of 2,500–7,000 ft; it is also recorded from Kunawar; from the Jhelum westwards it is reported from Malakand (the Kagan Valley) at 6,000 ft.

In Kashmir, it grows at Keran, Kishtwar, Marwa Dachhan, Ramban and Muzaffarabad. In the inner arid valleys of almost all the Punjab rivers it occurs usually growing on dry slopes; in Hazara it occurs on the banks of dry ravines.

Its common associates in the inner valleys, where it is more or less gregarious in dry localities, are *Quercus Ilex* and *Fraxinus xanthoxyloides*.

(iii) *Acer villosum* Wall., is a large tree of the Western Himalayas, which occurs between the Jhelum and Kumaon at altitudes of 7,000–9,000 ft. It does not seem to grow in Kagan, the Murree Hills or Kashmir but Hooker (1875, Vol. I, p. 695) records it from Kashmir (he does not mention any locality).

It is recorded from Jaunsar (altitude 8,000 ft.) Simla, the Ganges Valley, Tehri Garhwal (altitude 9,000–10,000 ft.) and Garhwal (alt. 9,000 ft.).

(iv) *Acer pictum* Thunb. is a handsome medium-sized tree, which occurs throughout the Himalayan ranges from the Indus to Bhutan at altitudes of 4,000–9,000 ft. It also occurs in the Trans-Indus territory and extends as far east as China and Japan. In Hazara it occurs at 8,000 ft., and also grows in the Murree Hills, where it is fairly common.

In Kashmir the species grows in Kamraj, the Kashmir Valley, Kishtwar, Marwa Dachhan, Ramban and the Sind Valley. It also occurs in Simla, Deoban, Jaunsur, Mussoorie and Landour.

(v) *Acer Cæsium* Wall. is the commonest maple of the Western Himalayas, which grows abundantly from Nepal westwards at altitudes of 7,000–10,000 ft.;

it may ascend to 12,000 ft. at some places and occasionally occurs at as low elevations as 4,000 ft.

In the mixed forests, where it is commonly associated with *Abies Pindrow, Pinus excelsa, Taxus baccata, Aesculus indica, Ulmus Wallichiana, Prunus Padus,* it often forms gregarious patches in shady places, or in valleys.

It is quite common in the Kagan Valley, Hazara, Chitral, the Murree Hills and the neighbouring Galis.

In Kashmir it grows abundantly at Sonamarg, the Kishenganga Valley, Kamraj, the Jhelum Valley, the Lidder Valley, Gurez, Keran, Kishtwar, Marwa Dachhan, Muzaffarabad, etc.

Along the outer Himalayan ranges this maple is the most widely distributed species occurring at Chamba, Bashahr, Jaunsar, Simla, Chakrata, Garhwal, Tehri Garhwal, the Pindari Valley, Kumaon, etc., etc.

The Species of *Acer* in the Modern Temperate Forests of the Western Himalayas

In the moist temperate deciduous forests the three Western Himalayan species of *Acer* occur gregariously in association with broad-leaved genera, *e.g.*, in Dwali, Western Almora Division (Kumaon in the United Provinces). *Acer Cæsium* and *Acer pictum* are found in association with *Aesculus indica, Carpinus viminea, Ulmus Wallichiana, Betula alnoides, Juglans regia, Fraxinus micrantha, Quercus semecarpifolia, Corylus colurna, Cornus macrophylla, Rhus punjabensis, Taxus baccata, Spiræa* (Champion, 1936, pp. 257–58).

In the Sutlej Valley (Punjab) we have the three species of *Acer*, namely *A. Cæsium, A. pictum, A. villosum* occurring together with *Aesculus indica, Betula alnoides, Fraxinus micrantha, Carpinus* sp., *Celtis australis, Juglans regia, Pyrus lanata, Ulmus* spp., *Abies Pindrow, Prunus cornuta, Corylus colurna, Cornus* spp., *Rhododendron arboreum, Rhus* sp., *Taxus baccata,* etc., etc. (Champion, *loc. cit.*, p. 258).

The same species of *Acer* aslo occur in Kulu and Hazara and the floristic composition of the forests is more or less similar to the forests at the above-mentioned places.

In the " Western oak-fir forests of Garhwal Himalayas " *Acer Cæsium* occurs with *Abies Pindrow, Picea Morinda* and several broad-leaved species, namely, *Quercus semecarpifolia, Q. dilatata, Ulmus Wallichiana, Aesculus indica, Corylus colurna, Rosa macrophylla, Rubus* sp., *Syringa Emodi,* etc. (Champion, *loc. cit.*, p. 245).

Acer Cæsium is associated with more or less the same species in the forests of Deoban (Chakrata division) also.

A few species of *Acer* occur in Pulga forests of the Parbatti Valley in Kulu and in the Sutlej Valley. In the former forests its associates are *Abies Pindrow, Pinus excelsa, Picea Morinda, Taxus baccata, Quercus semecarpifolia, Corylus colurna, Pyrus lanata* and species of *Rosa, Viburnum, Rubus, Spiræa,* and in the latter place the most common species occurring with them are *Abies Pindrow, Quercus semecarpifolia, Betula alnoides, B. utilis, Rhododendron campanulatum, Cotoneaster* sp., etc. (Champion, *loc. cit.*, pp., 245–46).

In the " western mixed coniferous forests of the Sutlej Valley " *Acer Cæsium, A. pictum* and *A. acuminatum* occur in association with the three most common Western Himalayan oaks, namely, *Q. incana, Q. dilatata* and *Q. semecarpifolia,* and several conifers including *Picea, Cedrus, Abies, Pinus excelsa* and *Taxus* (Champion, *loc. cit.*, p. 242).

In the " western mixed conifer forests of Grahan Nal, Parbatti Valley (Punjab) " *Acer* spp. occurs with *Abies, Picea, Cedrus, Juglans regia, Corylus colurna, Celtis australis, Ulmus Wallichiana* and here the oaks are not associated with maples (Champion, *loc. cit.*, p. 243). Similarly, in the broad-leaved forests of the Kashmir Valley, where the species of *Acer* are fairly abundant, we do not have a trace of oaks but the former are associated with *Populus ciliata, Juglans regia, Aesculus indica, Salix Wallichiana, Ulmus Wallichiana,* etc., etc.

" *Quercus dilatata–Acer* forests " are common in the Central Himalayas at an altitude of 7,000–8,000 ft. A little above the elevation zone of *Quercus incana, e.g.*, in Garhwal Himalayas *A. Cæsium* occurs with the three common species of oaks, *Betula alnoides, Carpinus viminea, Machilus Duthiei, Rhamnus purpurea,* etc. (Champion, *loc. cit.*, p. 235).

Past Distribution of the Genus *Acer*

Although an exhaustive account of the past distribution of the family is outside the scope of this paper, it may perhaps be useful to write a few lines regarding the existence of the Aceraceæ in time.

Fossil leaves and fruits belonging to several species of *Acer,* either closely related or identical with modern species, have been discovered from the Tertiary rocks of Europe and North America. In the Arctic regions they have occurred in a fairly good number during the Tertiary Period and southwards the genus was comparatively better represented then than to-day. From the geographical distribution of maples during the Tertiary times it may be conjectured that the genus *Acer* was probably more widely spread in the past than at the present time.

Maple Forests of the Kashmir Valley during the Pleistocene

The maples seem to have been more widely spread in the Valley during the Pleistocene than at the present time; out of a total of ten species recognised from the Karewas only three—*Acer Cæsium*, *A. acuminataum* and *A. pictum* are now seen in the Valley. The quantitative representation of the fossil species goes to show that the *Acer* spp. had occupied a fairly prominent position in the vegetation of that time and they might have formed extensive belts in forests of broad-leaved trees.

Acer oblongum, a tropical species of the Sub-Himalayan zones, which occurred on the northern slopes of the Pir Panjal Range at Liddarmarg during the Pleistocene, probably in association with several broad-leaved species, namely, *Machilus odoratissima*, *M. Duthiei*, *Phœbe lanceolata*, *Ficus Cunia*, *Buxus papillosa*, *B. Wallichiana*, *Mallotus phillipinensis*, in forests of *Quercus incana* and *Quercus glauca*, has now totally disappeared from these regions since the early Pleistocene.

Of the two species—*A. pentapomicum*, and *A. pictum* and other incompletely determined species of *Acer*, which during the Pleistocene, were probably associated with *Ulmus Wallichiana*, *Ulmus lævigata*, *Aesculus indica*, *Carpinus faginea*, *Alnus nitida*, *Rosa macrophylla*, *Quercus dilatata*, *Q. Ilex*, *Viburnum cotinifolium*, *Engelhardtia Colebrookeana*, etc., etc., at Laredura, only one (*A. pictum*) is still present in the valley; the other species has now taken to more favourable climatic regions.

Of the other two species, namely, *A. villosum* and *A. Cæsium*, which occurred at Ningal Nullah in an altogether different association, composed of *Salix Wallichiana*, *S. denticulata*, *Salix* spp., *Populus ciliata*, *Populus nigra*, *Rhamnus purpurea*, *Juglans regia*, *Aesculus indica*, *Pyrus Malus*, etc. *A. villosum* has again disappeared from the Kashmir Valley. The flora at this locality (Ningal Nullah) during the Pleistocene was characterised by the absence of oaks, laurels, figs, box, etc., which are tropical species and in this respect it has a good deal in common with the modern flora of the Kashmir Valley adjoining the northern slopes of the Pir Panjal Range.

Summary

1. The paper describes about ten species of the Aceraceæ five of which are completely determined; the remaining include four leaf species, which do not match any modern species of the Himalayas; one species, *viz.*, *A. Cæsium* is based on a few fragments of fruits, the other bits of samaras could not be specifically determined by comparison with any modern species.

2. The modern distribution of the Aceraceæ brings to light the absence from the Kashmir Valley of three species (*A. oblongum*, *A. penta-*

pomicum and *A. villosum*) which occur in the fossil state in the Karewa beds. However, the other two species, namely *A. Cæsium* and *A. pictum* are important constituents of the broad-leaved forests of the Valley as well as the northern slopes of the Pir Panjal Range. The modern distribution of the fossil species in the Himalayan regions is given together with their different associates in different types of forests.

3. A comparison of the modern maple forests of the Kashmir Valley with those of the Pleistocene period shows that the oaks and laurels, which must have occurred during the Pleistocene in association with maples, are altogether absent from the valley at the present time. This fact shows that the climate of the valley has changed since the early Pleistocene times.

4. The inclusion in the fossil flora of the Aceraceæ does not alter the general conclusions already expressed by the author regarding the changes of climate and altitude of the Valley since the early Pleistocene times.

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Explanation of Plates

All the photographs in Plates XXIII–XXVIII are from untouched negatives. Figured specimens excepting those of *Acer oblongum* (Figs. 1-5), which are the property of the Geological Survey of India, are preserved in the Botany Museum, University of Lucknow. (Sahni Collection).

Plate XXIII. *Acer oblongum* Wall.

Fig. 1. Leaf (impression of the upper surface). Middlemiss collection, G.S.I. No. K 14/948 *a*, Liddarmarg. Nat. size.

Fig. 2. Leaf (impression of the lower surface) showing tri-nerved base. Middlemiss collection, G.S.I. No. K 14/948 *a* 23, Liddarmarg. Nat. size.

Fig. 3. Leaf (impression of the upper surface). Middlemiss collection, G.S.I. No. K 14/948 *a*, 13, Liddarmarg. Nat. size.

Fig. 4. A part of the leaf (marked × in Fig. 3) enlarged to show loops of the laterals; tertiary and finer reticulations. × *Ca.* 5.

Plate XXIV

Acer oblongum Wall.

Fig. 5. Leaf (impression of the lower surface). Middlemiss collection, G.S.I. No. K 14/948 *a*, 9, Liddarmarg. Nat. size.

Acer pentapomicum J. L. Stewart

Fig. 6. Leaf impression. G. S. Puri collection, Loc. 1 N 6, Ningal Nullah. Nat. size.

Fig. 7. A part of the leaf (marked × in Fig. 6) enlarged to show nature of the laterals; tertiary and finer reticulations. ×*Ca.* 5.

Fig. 8. Leaf impression. de Terra collection, Loc. 3 L 16″, Liddarmarg. Nat. size.

Acer villosum Wall.

Fig. 9. Leaf (impression of the upper surface). R. R. Stewart collection, L 193, Laredura. Nat. size.

Fig. 10. A part of the leaf (marked ×× in Fig. 9) enlarged to show nature of the laterals; tertiary and finer reticulations. × *Ca.* 5.

PLATE XXV

Acer pictum Thunb.

Fig. 11. Leaf (impression of the lower surface). G. S. Puri collection, Loc. 1 N 7, Ningal Nullah. Nat. size.

Acer Cæsium Wall.

Fig. 12. Wing of a fossil samara, R. R. Stewart collection, L 160, Laredura. Nat. size.

Fig. 13. Winged nut of a modern double samara for comparison with the fossil (Fig. 12).

Acer sp.

Fig. 14. Samara (one half) showing the basal nut and wing. de Terra collection, Loc. 3 L 50, Liddarmarg. Nat. size.

Fig. 15. Counterpart of Fig. 14. Loc. 3 L 51, Liddarmarg. Nat. size.

Acer sp. A

Fig. 16. Leaf (impression of the upper surface). G. S. Puri collection, L 670, Laredura. Nat. size.

Fig. 17. A part of the leaf (marked ×× in Fig. 16) enlarged to show nature of the laterals; tertiary and finer reticulations. ×*Ca.* 5.

Acer sp.

Fig. 18. Samara. G. S. Puri collection, L 720, Laredura. Nat. size.

Fig. 19. Samara. G. S. Puri collection, L 721, Laredura. Nat. size.

Fig. 20. Samara. G. S. Puri collection, L 549, Laredura. Nat. size.

PLATE XXVI. *Acer* sp. B

Fig. 21. Leaf (impression of the upper surface). G. S. Puri collection, L 547, Laredura. × *Ca.* ½ Nat. size.

Fig. 22. One lobe of the leaf (marked 1 in Fig. 21) enlarged to show the loops of the laterals and inframarginal vein. ×*Ca.* 4.

Fig. 23. A part of the leaf (marked ×× in Fig. 21) enlarged to show tertiary and finer reticulation. ×*Ca.* 5.

PLATE XXVII. *Acer* sp. C

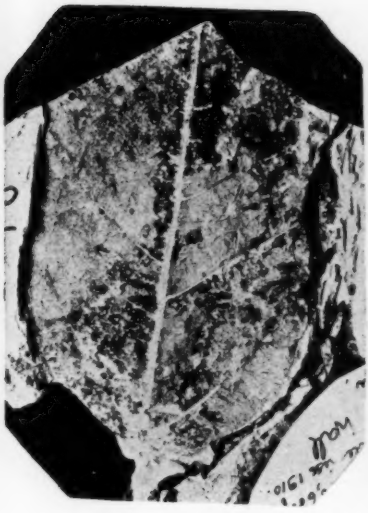
Fig. 24. Leaf impression. G. S. Puri collection, Loc. 1 N 8, Ningal Nullah. Nat. size.

Fig. 25. A part of the leaf (marked ×× in Fig. 24) enlarged to show tertiary and finer reticulations. ×*Ca.* 3.

PLATE XXVIII. *Acer* sp. D

Fig. 26. Leaf (impression of the upper surface). G. S. Puri collection, L 803 *a*, Laredura. Nat. size.

Fig. 27. A part of the counterpart of the leaf (Fig. 26) showing tip of a lobe. L 803 *b*. Nat. size.



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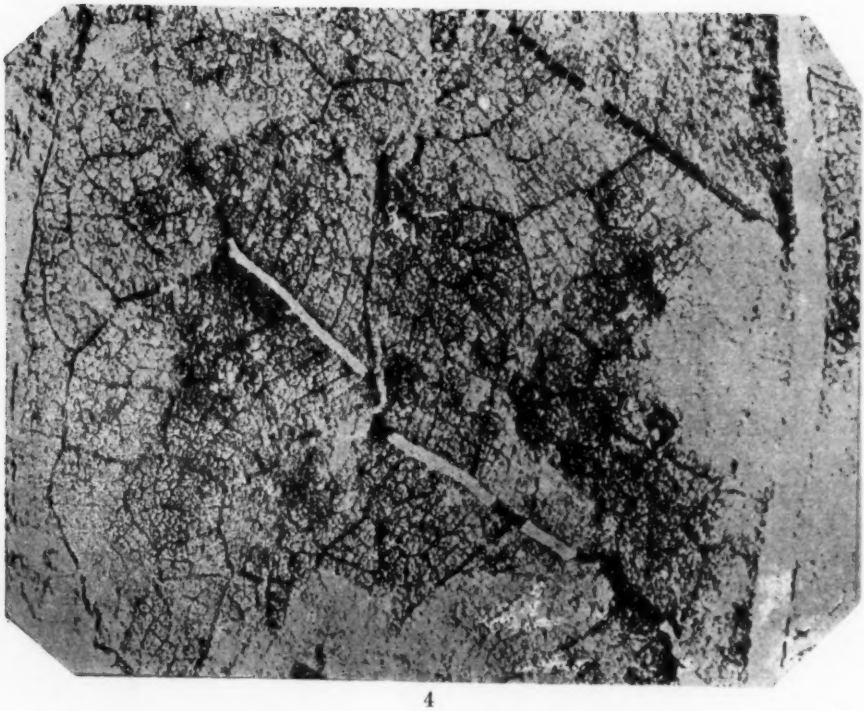

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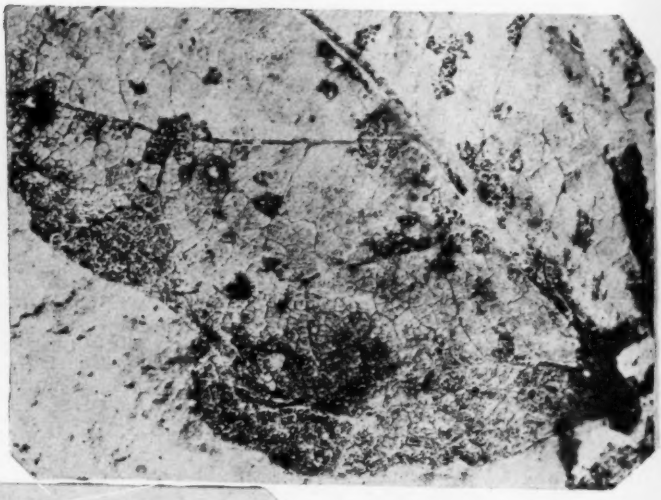

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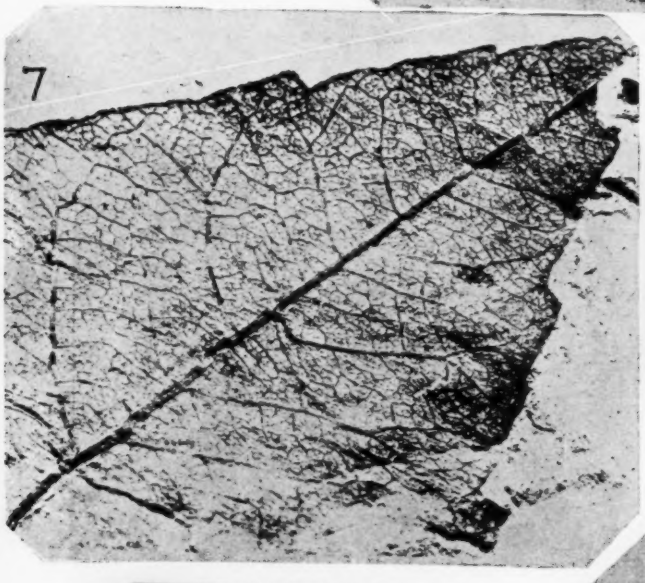

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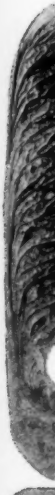

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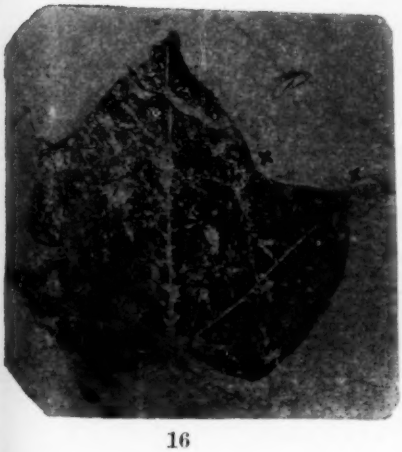

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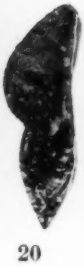

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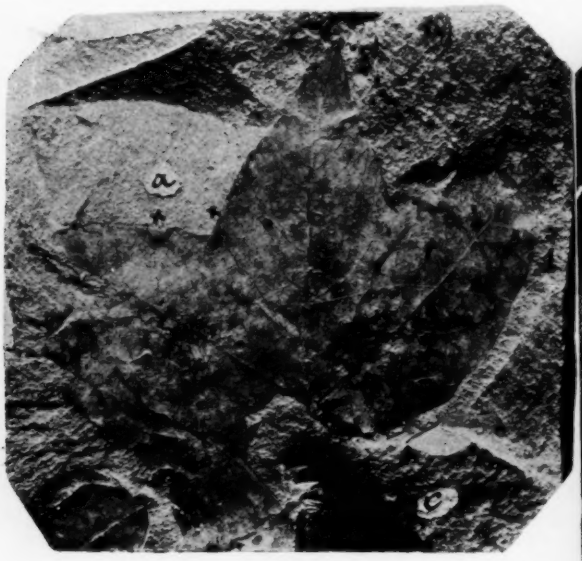

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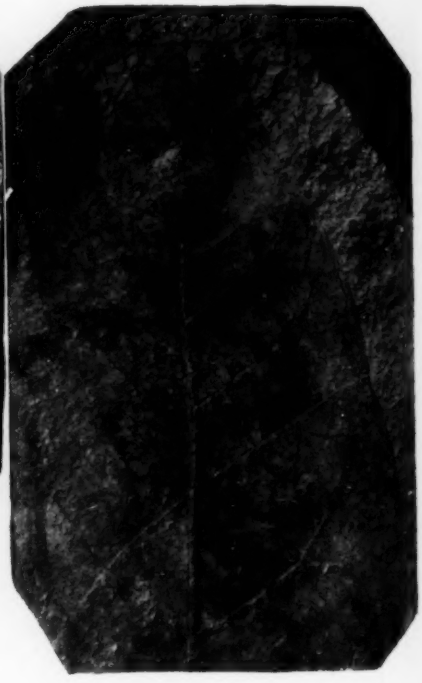

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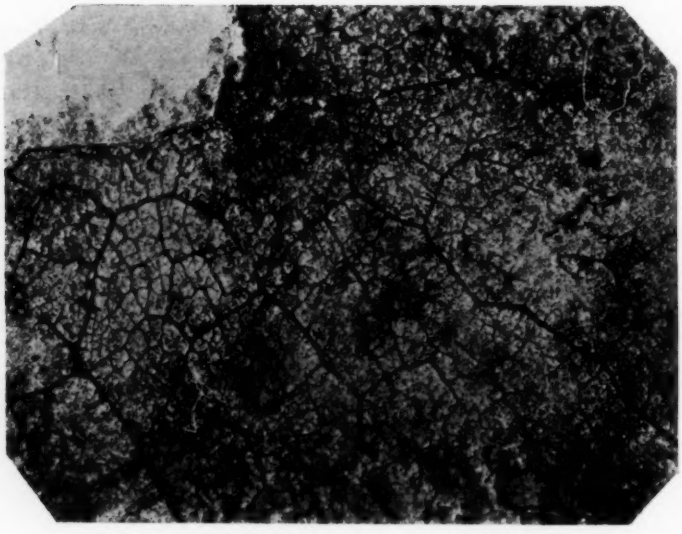

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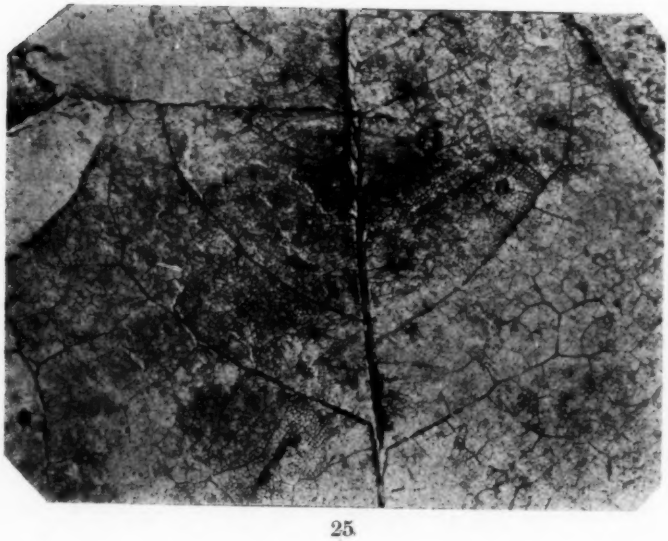

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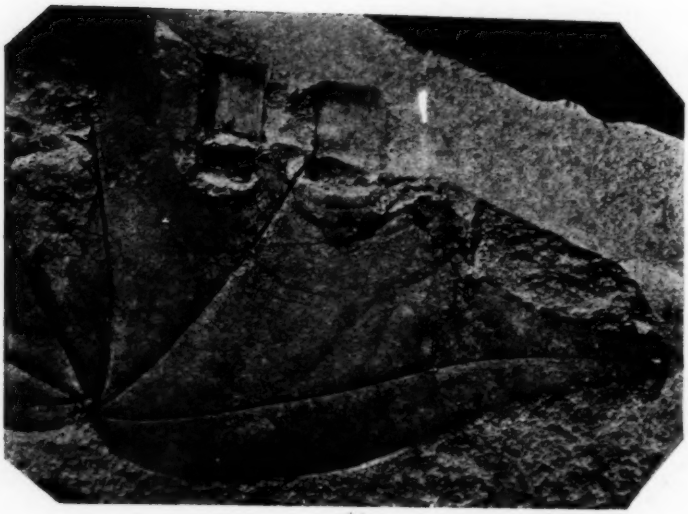

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OSTEOMETRIC SEX DETERMINATION FROM THE HEAD AND LOWER END OF A FEMUR

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Received July 24, 1945

(Communicated by Prof. Inderjit Singh, F.A.SC.)

Figures worked out from European femora are available for osteometric determination of sex, from the head and lower end of the bones (Parson, 1913; Frazer, 1937). As no such figures were available for Indian bones, and the matter being of some medico-legal importance, an attempt was made to arrive at figures, applicable to Indians, by which sex could be determined by examination of the head and lower end of femora osteometrically.

Material and Method

A series of adult, fresh femora of definitely known sex were examined. Apparently diseased bones were rejected. The source of material, and the instruments used, were the same as for a previous communication (Siddiqui and Shah, 1944). In all, 185 bones were examined, of which 146 were from known male bodies. The number of femora from known female bodies (39), was admittedly small, but no more could be collected in five years (1940–45). The bones being entirely from Punjabis the result would be primarily applicable to these people.

Osteometry

1. *Head of the Femur*

(*a*) *Maximum diameter.*—This was measured by moving the jaws of the slide compass all round the head till the scale registered the maximum reading. This was found usually at right angles to the long axis of the neck of the femur.

(*b*) *Minimum diameter.*—This was also measured by rotating the slide compass all round the head till minimum reading was recorded. This was most often the vertical diameter of the head.

TABLE I

Sex	Max. Diameter in mm.	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Male 146	No. of bones							8	8	10	22	24	16	26	8	4	6	6	2	3	2	0	1
	Percentage							5·4	5·4	6·8	15·0	16·4	10·9	17·8	5·5	2·7	4·1	4·1	1·3	2·0	1·3	0	0·6
Female 39	No. of bones	1	1	3	4	8	5	10	2	5													
	Percentage	2·5	2·5	7·7	10·2	20·5	12·8	25·6	5·1	12·8													

TABLE II

Sex	Min. Diameter in mm.	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Male 146	No. of bones							12	4	18	20	24	22	22	4	2	13	1	4
	Percentage							8·2	2·7	12·3	13·6	16·4	15·0	15·0	2·7	1·3	8·9	0·6	2·7
Female 39	No. of bones	1	1	6	3	6	3	11	4	2	2								
	Percentage	2·5	2·5	15·3	7·7	15·3	7·7	28·2	10·2	5·1	5·1								

TABLE III

Sex	Width in mm.	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
Male 106	No. of bones ..									2	6	3	6	6	14	10	10	10	8	14	4	8	2	2	0	1
	Percentage ..									1·8	5·6	2·8	5·6	5·6	13·2	9·4	9·4	9·4	7·5	13·2	13·2	7·5	1·8	1·8	0	0·9
Female 39	No. of bones ..	6	0	0	9	6	0	0	6	6	3	3														
	Percentage ..	15·3	0	0	23·0	15·3	0	0	15·3	15·3	7·7	7·7														

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2. *Lower End of the Femur*

The maximum width between the two condyles was measured. The prongs of the slide compass were kept in touch with the lateral and medial margins of the respective femoral condyles, so as to exclude the epicondyles.

Results

The measurements of the head of femur are given in Tables I and II, and that for the lower end in Table III.

Examination of Table I makes it clear that, in the case of males the maximum diameter of the head was, in no bone, below 41 mm. and in the case of females in no bone above 43 mm. Therefore, the bones with maximum diameter of the head above 43 mm. would appear, most likely, to be those of males, and bones with corresponding diameter below 41 mm. similarly of females. As, however, the total number of bones examined was not large enough, a millimeter of error may be allowed in either figure. It could thus be safely stated that the maximum measurement of the head of a femur, in male, is above 44 mm. and that in the female below 40 mm. Those with this diameter lying between the figures 40 mm. and 44 mm. may belong to either sex, and osteometry is of no help. In such bones other sexual features have to be considered.

If the minimum diameter of the head be taken (Table II) results appear substantially the same as for the maximum diameter. Bones with this diameter above 43 mm. belong to males, and those below 40 mm. to females. Corrected figures for males would be above 44 mm. and for females below 39 mm. Those with measurements between these extremes may be grouped as doubtful, for they may be of either sex.

Table III shows that in the case of males the maximum width of the lower end was in no bone, below 67 mm. and in the case of females no bone above 69 mm. The conclusion is that a femur with this measurement above 69 mm. is, most likely to be that of a male, and that below 67 mm. to be a female. After allowing a margin of 1 mm. error, the corrected figure for the males would be above 70 mm. and for the females below 66 mm. Doubtful bones would be those with measurements between 66 mm. and 70 mm.

Corresponding comparative figures of English and Indian bones are given in Table IV for use of medico-legal officers.

TABLE IV

Measurement taken	Country	Male	Female	Doubtful
Maximum Diameter Head	England—Frazer (1937)	Above 46 mm.	Below 42 mm.	42 mm. to 46 mm.
	Present authors (1945)	Above 44 mm.	Below 40 mm.	40 mm. to 44 mm.
Max. Width Lower End	England—Frazer (1938)	Not available	Below 71 mm.	Not available
	India—Present authors (1945)	Above 70 mm.	Below 66 mm.	66 mm. to 70 mm.

SUMMARY

Osteometric evaluation of the maximum and minimum diameters of the head, and of the maximum width of the lower end of femur in 185 fresh adult, Punjabi femora (146 male and 39 female) seems to point to a method of determining sex from these bones which could be employed with advantage by medico-legal workers. Although the series presented is small and does not admit of wide generalisations the following conclusions appear obvious:—

1. The Punjabi femora with minimum and/or maximum diameters of the head above 44 mm. and the width of the lower end above 70 mm. belong to males.

2. In the case of females the corresponding diameters are below 40 mm. and 66 mm. respectively.

3. The bones with measurements of the head lying between 40 mm. and 44 mm. and of the lower end between 66 mm. and 70 mm. may be of either sex. In such cases other sexual features in these bones would have to be considered for determining the sex.

ACKNOWLEDGMENT

One of us (M.A.S.) is indebted to Professor M. A. H. Siddiqui, Head of the Department of Anatomy, King Edward Medical College, Lahore, from whose Department the material for investigation under report was collected (1940–45), while working in that institution.

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AMINOACID DEAMINATION BY THERMOPHILIC BACTERIA—PART I

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Received July 5, 1945

The studies on bacterial deamination have in general not been carried out as widely as studies on tissue deamination. Harden (1901) reported the observation that *Bacteria coli* cultures in glucose broth deaminate aspartic acid. Quastel and Woolf (1926) showed that *Bact. coli* could bring about the following reactions:

(1) $COOH.CH_2.CH.NH_2.COOH \rightarrow COOH.CH_2.CH_2.COOH + NH_3$
Aspartic acid — Succinic acid

(2) $COOH.CH_2.CH.NH_2.COOH \rightarrow COOH.CH=CH.COOH + NH_3$
Aspartic acid — Fumaric acid

The first of these reactions takes place anaerobically and in the absence of inhibitors whereas in the presence of inhibitors the deamination of aspartic acid proceeds according to the second equation. Virtanen and Tarnanen (1932) found that *Bacteria fluorescens liquefaciens* could deaminate aspartic acid, the optimum pH was 7·0 to 7·5 and no other aminoacid was deaminated. Strickland (1934) has studied the anaerobe *Clostridium sporogenes* and has found that its suspensions deaminate alanine optimally at pH 7·5. Bernheim *et al.* (1935) examined the deamination of aminoacids by resting cell suspensions of *B. proteus*. Webster and Bernheim (1936) have studied the oxidation and deamination of a number of aminoacids by *B. pyocyaneus*. Janke and Tyenthal (1936) have found that *B. coli, B. vulgar, B. mycoides,* and *Pseudomonas flourescens* oxidatively deaminate glycine. Woods and Cliften (1937) report that *Clostridium tetanomorphum* anaerobically deaminates as well as decarboxylates a large number of aminoacids. Stephenson and Gale (1937), Gale and Stephenson (1938) and Gale (1938) have also made a comprehensive study of the deaminating activity of *B. coli*; the optimum pH for deamination of various aminoacids, the effect of addition of various compounds and the decay of activity of washed suspensions was studied. Hills (1940) has studied aminoacid deamination by certain bacteria, while Desnuelle and Fromageot (1939) have studied the anaerobic bacterial degradation of cystine and cysteine.

The present study is concerned with deamination of aminoacids by thermophilic bacteria. These bacteria have the peculiar property of growing

at high temperatures and this renders them an intriguing subject for study. They are fairly widely distributed in soil (Glebig, 1888; Rabinowitsch, 1895; Weinzirl, 1919; and Bergey, 1919), and undoubtedly play a part in several soil and agricultural reactions. In Beccari system of garbage fermentation temperatures above 50° C. are reached and thermophilic bacteria participate in the liquefaction of proteins in the first stage and in the decomposition of the resulting aminoacids in the second stage. In the thermogenic fermentation of tobacco, aminoacids are first formed from proteins and are then deaminated.

Methods of Analysis and General Experimental Methods

In most of the experiments here described the organisms were used in the form of washed suspensions. The bacteria were grown in nutrient broth, reaped by centrifuging at 3,000 r.p.m. for fifteen minutes and washed once with distilled water. The bacterial mass was then made into an aqueous suspension which was rendered as homogeneous as possible by shaking. Equal volumes of this suspension withdrawn after shaking produced nearly the same amount of deamination.

For most experiments it was not necessary to determine the quantity of cells per ml. of the suspension. Where experiments had to be made to compare the relative deaminating ability of cells cultivated in different ways, it was necessary to standardise the suspensions. For this purpose an aliquot volume of the suspension was filtered through a tiny porcelain filter fitted into the side arm of a microbeaker; all the cells were retained in the beaker and their weight was determined by difference after washing with distilled water and desiccating the beaker and contents *in vacuo* over phosphorous pentoxide and caustic soda at room temperature to constant weight. The average deviation in values obtained in duplicate estimations of this nature was less than 3·3% and the differences in deaminating ability of cells grown in different ways, where significant, were at least 50 to 100%. This gravimetric process of standardisation had to be adopted because the suspensions, although macroscopically uniform, still contained aggregates of large number of cells which settled under gravity rather too rapidly to allow of accurate results being obtained by nephelometric methods, besides the disaggregation of the cells in the suspension which also determines the opacity could never be always reproduced. Counting of cells either after staining or on plating also gave inaccurate results for similar reasons. Nephelometric methods and counting procedures could only be applicable in cultures whose age was twenty-four hours or less, because in such cultures the cells had not yet aggregated themselves into film-like structures.

Deamination of aminoacids can be quantitatively followed by estimating either any of the reactants or any of the products of reaction. Were it not for complications due to side reactions estimation of oxygen consumed, ketoacid or other nitrogen-free acid formed, loss of α-amino nitrogen, or ammonia produced could all be used for quantitatively studying deamination. Although suspensions of what may be called " resting cells " were used in the present study, there is always the possiblity that oxygen is being consumed both in the presence and in the absence of aminoacids in reactions quite distinct from pure deamination. The ketoacids produced are often degraded further or used up in the formation of more complex carbohydrate like substances in a manner which is only imperfectly understood at present. On account of these limitations estimation of ammonia produced or α-amino nitrogen disappeared gives the best possible approximation of the extent of deamination. It is unlikely that the ammonia liberated enters into any side reactions and if there is no significant back reaction then the whole of the ammonia produced will be present as such in the reaction mixture. During the investigations on bacterial deamination here described attempts were made to reverse the process of deamination, for example in case of aspartic acid by starting with succinic, oxalacetic or fumaric acids and ammonium chloride but no decrease in free ammonia could be detected at all the hydrogen-ion concentrations. Errors due to back reaction may therefore be ruled out. The rate of ammonia formation in an aminoacid bacterial cell mixture, corrected for the blank value due to autodeamination of the cells themselves therefore represents very nearly the actual rate of deamination of the aminoacid. In the present experiments the rate of formation of free ammonia corresponded to the rate of decrease of α-aminoacids as measured in the Van Slyke apparatus. Both methods gave comparable results, but the former method was standardised for the purpose of detailed study of deamination. Estimation of ammonia was carried out in a microkjeldahl apparatus of the Parnas type. The reacting mixture was brought to pH 10–11 and then steam-distilled for three to five minutes, the distillate being received either in M/100 sulphuric acid or in a saturated solution of boric acid as usual.

The experimental technique employed was very simple. The organisms were cultivated in nutrient broth by incubating at their respective optimum temperatures. The cells were collected by centrifuging the cultures at 3,000 r.p.m. for fifteen minutes. After washing once in the centrifuge the bacterial mass was resuspended in a known volume of distilled water and the suspension made homogeneous by shaking. Known volume of the suspensions and the aminoacid solution made at the stated pH in McIlvaine's

citrate-phosphate buffer were mixed and the increase in free ammonia was estimated after incubation at 40° C. for stated lengths of time. Careful blank experiments were performed simultaneously and proper corrections for auto-deamination of the cells were made. Where delay in carrying out the estimation was unavoidable the reaction was stopped after the stated period by adding 0·100 ml. of 4 M hydrochloric acid to every 10 ml. of the mixture of aminoacid solution and bacterial suspension. This also fixed up the free ammonia so that no loss could take place. The reactions were all carried out in either stoppered hard-glass test-tubes or conical flasks.

Deamination of dl-*aspartic acid,* dl-*alanine, glycine,* dl-*glutamic acid and* dl-*leucine*

The bacteria were cultivated in nutrient broth by incubation at 50° C. for 48 hours and were reaped by centrifuging. The cells were washed and made up into a uniform suspension in distilled water. 5 ml. of this suspension were added to 20 ml. of M/66 solution of the aminoacid made at the required pH in McIlvaine's citrate-phosphate buffer and the mixture was shaken. A 5 ml. portion of the mixture was withdrawn immediately and analysed for ammonia content. The remainder was incubated for 24 hours at 40° C. in well stoppered test-tubes after which the reaction was stopped by adding a drop of pure hydrochloric acid and ammonia estimation was again carried out in duplicate. Blank experiments were conducted at all hydrogen-ion concentrations in two sets. In the first set the bacterial suspension was replaced by 5 ml. of distilled water and in the second the aminoacid solution was replaced by an equal volume of distilled water. The data reported below have been corrected for the two blank values.

The optimum pH for the deamination of *dl*-aspartic acid by *Bacillus thermophilus, B. œrothermophilus* and *B. thermoacidurans* is, therefore 7·7, 7·7 and 8·0 respectively, and for *dl*-alanine 8·4, 7·9 and 8·1 respectively; glycine is deaminated optimally at pH 7·9, 7·5 and 8·3 respectively; *dl*-glutamic acid at pH 7·2, 7·5 and 8·0; and *dl*-leucine at pH 7·4, 7·2 and 7·9 respectively.

Relative rate of deamination of dl-*aspartic acid,* dl-*alanine,* dl-*glutamic acid, glycine and* dl-*leucine*

In order to determine the relative ease with which the various aminoacids are deaminated equal volumes of the different bacterial suspensions were added to solutions of the different aminoacids prepared at their respective pH optimas for deamination. The amount of ammonia liberated on incubation was determined as usual and corrected for blanks. The results are summarised in Table II.

TABLE I

Ammoniacal nitrogen liberated in 24 hours, mgm./5 ml. of cell suspension

pH	*B. thermophilus*	*B. aerothermophilus*	*B. thermoacidurans*
	dl-*Aspartic acid*		
6·5 ..	0·203	0·193	0·163
7·2 ..	0·492	0·410	0·295
7·9 ..	0·662	0·586	0·513
8·6 ..	0·440	0·402	0·405
9·3 ..	0·180	0·175	0·232
10·0 ..	0·076	0·008	0·071
	dl-*Alanine*		
6·5 ..	0·170	0·144	0·105
7·2 ..	0·215	0·372	0·238
7·9 ..	0·300	0·506	0·495
8·6 ..	0·523	0·370	0·442
9·3 ..	0·283	0·130	0·201
10·0 ..	0·092	0·065	0·072
	Glycine		
6·5 ..	0·105	0·104	0·095
7·2 ..	0·189	0·386	0·206
7·9 ..	0·286	0·384	0·368
8·6 ..	0·178	0·112	0·372
9·3 ..	0·108	0·080	0·195
10·0 ..	0·040	0·006	0·082
	dl-*Glutamic acid*		
6·5 ..	0·172	0·125	0·063
7·2 ..	0·286	0·248	0·125
7·9 ..	0·170	0·242	0·205
8·6 ..	0·105	0·128	0·172
9·3 ..	0·062	0·062	0·092
10·0 ..	0·005	0·015	0·022
	dl-*Leucine*		
6·5 ..	0·195	0·201	0·182
7·2 ..	0·482	0·325	0·281
7·9 ..	0·366	0·208	0·314
8·6 ..	0·212	0·116	0·286
9·3 ..	0·085	0·032	0·161
10·0 ..	0·010	0·000	0·008

TABLE II

Aminoacid	Ammoniacal nitrogen liberated in 24 hours by the different bacteria, mgm./5 ml. of cell suspensions		
	B. thermophilus	*B. aerothermophilus*	*B. thermoacidurans*
dl-Aspartic acid ..	0·810	0·630	0·700
dl-Alanine ..	0·460	0·370	0·390
Glycine ..	0·300	0·265	0·280
dl-Glutamic acid ..	0·305	0·350	0·260
dl-Leucine ..	0·665	0·650	0·625

It is therefore obvious that the relative ease of deamination of the different aminoacids does not vary from bacteria to bacteria under study. *dl*-Aspartic acid and *dl*-leucine are always the easiest to deaminate followed by *dl*-alanine, while glycine and *dl*-glutamic acid are the most difficult to deaminate.

Effect of anaerobic conditions on the deamination of dl-*aspartic acid*

In order to find out whether deamination requires the presence of oxygen parallel experiments were conducted under atmospheric conditions and in an hydrogen atmosphere. For experiments under hydrogen atmosphere the aminoacid solutions were deaerated thoroughly by vacuum and bubbled through with pure hydrogen. This process was repeated thrice. The bacterial suspension was also deaerated, measured out and mixed rapidly with the aminoacid solution, the mixture again deaerated and bubbled through with pure hydrogen after which the flasks were sealed off and incubated. Proper corrections were made for blanks. The aminoacid used was *dl*-aspartic acid.

TABLE III

Organism	Ammoniacal nitrogen liberated in 24 hours under atmospheric conditions, mgm./10 ml. of mixture	Ammoniacal nitrogen liberated in 24 hours under hydrogen atmosphere, mgm./10 ml. of mixture
B. thermophilus ..	0·300	0·105
B. acrothermophilus	0·245	0·070
B. thermoacidurans	0·215	0·005

Deamination by these organisms is therefore an oxidative process and is restricted by absence of oxygen. The slight apparent increase in ammoniacal nitrogen under hydrogen atmosphere is probably due to traces of oxygen which could not be driven out in the process of deaeration and bubbling through with hydrogen.

Effect of presence of particular aminoacids in growth medium on the deaminating capacity of cells grown therein

If bacterial enzyme systems are adaptive in nature then it may be expected that if an aminoacid is added to the medium in which the bacteria are cultivated then the ability of cells obtained from such cultures to deaminate the particular aminoacid will be greater as compared to the deaminating ability of cells which have been grown in media without the aminoacid. This question was examined in case of *dl*-aspartic acid and *dl*-alanine with all the three bacteria and the results are summarised in Table IV. Cells obtained from nutrient broth containing *dl*-aspartic acid were tested for deaminating

ability against *dl*-aspartic acid alone, those obtained from broth containing *dl*-alanine were tested against *dl*-alanine only and cells obtained from plain broth were tested, for comparison, both against *dl*-alanine and *dl*-aspartic acid. Blank corrections were made for any increase in ammoniacal nitrogen in absence of the bacterial cells and for auto-deamination of the cells.

TABLE IV

Organism	Nature of medium	Mgm. dry wt. of the suspension per 5 ml.	Mgm. ammoniacal nitrogen liberated by 5 ml. of the suspension		Mgm. ammoniacal nitrogen liberated per 10 mgm. dry wt. of cells	
			dl-Aspartic acid	*dl*-Alanine	*dl*-Aspartic acid	*dl*-Alanine
B. thermophilus ..	Plain nutrient broth	12·66	0·667	0·532	0·527	0·420
	Nutrient broth with 1% *dl*-aspartic acid	13·89	0·987	..	0·718	..
	Nutrient broth with 1% *dl*-alanine	10·15	..	0·886	..	0·872
B. aerothermophilus	Plain nutrient broth	14·15	0·596	0·515	0·421	0·364
	Nutrient broth with 1% *dl*-aspartic acid	10·98	0·685	..	0·624	..
	Nutrient broth with 1% *dl*-alanine	13·20	..	0·697	..	0·528
B. thermoacidurans	Plain nutrient broth	18·78	0·823	0·718	0·438	0·382
	Nutrient broth with 1% *dl*-aspartic acid	16·85	1·057	..	0·627	..
	Nutrient broth with 1% *dl*-alanine	12·16	..	0·965	..	0·793

It is obvious from Table IV that in case of the three thermophilic bacteria presence of any aminoacid in the medium in which the bacteria were grown increases their ability to deaminate this particular aminoacid.

Effect of oxidation-reduction reagents on deamination

It has been mentioned before that deamination of aminoacids by washed suspensions of the thermophilic bacteria is probably an oxidative process because it is suppressed by the careful exclusion of oxygen. Hydroquinone was found, as expected, to suppress the course of deamination of *dl*-aspartic acid by all the four bacteria. Methylene blue was rapidly reduced to the leuco base by mixtures of cell suspensions and the aminoacid, the cells alone possessed only slight capacity to reduce the methylene blue. This shows that

the aminoacid serves directly or indirectly as the hydrogen donating system. In spite of this the speed of deamination was not accelerated by the presence of methylene blue. Care was taken in all these experiments to ensure that the optimum pH was not altered by the addition of methylene blue or hydroquinone. The aminoacid employed was *dl*-aspartic acid at optimum pH.

TABLE V

Effect of hydroquinone on deamination

Organism	Concentration of hydroquinone in the mixture	Mgm. of ammoniacal nitrogen liberated per 5 ml. of cell suspension
B. thermophilus ..	Nil	0·537
	M/200	0·132
B. aerothermophilus ..	Nil	0·519
	M/200	0·337
B. thermoacidurans ..	Nil	0·425
	M/200	0·234

TABLE VI

Effect of methylene blue on deamination

Organism	Concentration of methylene blue in the mixture	Mgm. of ammoniacal nitrogen liberated per 5 ml. of cell suspension
B. thermophilus ..	Nil	0·532
	M/550	0·547
	M/250	0·543
B. aerothermophilus ..	Nil	0·521
	M/550	0·501
	M/250	0·473
B. thermoacidurans ..	Nil	0·435
	M/550	0·411
	M/250	0·389

Effect of narcotics on bacterial deamination

Chloroform and toluene were the two reagents tested. 0·5 ml. of each was added to each mixture consisting of 5 ml. washed cell suspension, 10 ml. M/40 solution of *dl*-aspartic acid and 10 ml. of buffer and the mixture was shaken. Controls received no toluene or chloroform.

Toluene and chloroform therefore destroy the deaminating capacity of freshly prepared suspensions of all three bacteria. The effect of narcotics on an old suspenison is however, not so striking.

TABLE VII

Organism	Mgm. of ammoniacal nitrogen liberated per 5 ml. of cell suspension		
	In absence of chloroform or toluene	In presence of chloroform	In presence of toluene
B. thermophilus—Fresh suspension ..	0·495	0·000	0·072
Do. 5-day old suspension ..	0·473	0·325	0·380
B. aerothermophilus—Fresh suspension ..	0·600	0·013	0·022
Do. 5-day old suspension ..	0·645	0·406	0·387
B. thermoacidurans—Fresh suspension ..	0·586	0·007	0·012
Do. 5-day old suspension ..	0·617	0·484	0·502

DISCUSSION AND SUMMARY

Broadly speaking biological deamination of aminoacids is generally of three types:

(1) Oxidative deamination:

$$R.CH(NH_2).COOH + O \rightarrow R.CO.COOH + NH_3$$

(2) Reductive deamination:

$$R.CH(NH_2).COOH + H_2 \rightarrow R.CH_2.COOH + NH_3$$

(3) Hydrolytic deamination:

$$R.CH(NH_2).COOH + H_2O \rightarrow R.CH(OH).COOH + NH_3$$

Washed suspensions of the thermophilic bacteria *Bacillus thermophilus*, *B. aerothermophilus* and *B. thermoacidurans* possess the property of deaminating a number of aminoacids, for example, aspartic acid, alanine, glycine, glutamic acid and leucine. This deamination is of the oxidative type because it is strongly suppressed by the exclusion of oxygen. This property of oxidative deamination makes these bacteria important factors in soil ammonia formation and in the various biological processes in which ammonia is formed from aminoacids, especially at relatively high temperatures, for example in garbage and silo fermentation, sewage disposal, etc. The optimum pH of deamination varies with the different bacteria under study and with the different aminoacids; but is always in the alkaline region. In natural processes where deaminating ability of these organisms is called into play the reaction of the medium is generally either neutral or slightly acidic. But as soon as a little ammonia has been produced the reaction would start getting more and more alkaline thus favouring the production of more ammonia. As was to be expected the capacity of these bacteria to deaminate any particular aminoacid is enhanced if that aminoacid is present in the growth medium. As the deamination brought about by these bacteria

is of oxidative type it is suppressed in the presence of an hydrogen donating reagent like hydroquinone; but contrary to expectations methylene blue does not favour deamination. Toluene and chloroform destroy the deaminating capacity of fresh cell suspensions of these bacteria but only partially suppress that of five-day old suspensions.

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AMINOACID DEAMINATION BY THERMOPHILIC BACTERIA—PART II

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Received July 5, 1945

The thermophilic bacteria are fairly widely distributed in soil, etc., and play a role in thermogenic fermentations. It would be interesting to investigate the aminoacid deaminating properties of these bacteria. The present study is devoted to the deaminating properties of *Bacillus thermophilus, B. aerothermophilus* and *B. thermoacidurans*. In Part I of this paper it has been shown that these bacteria oxidatively deaminate a number of aminoacids in an alkaline pH. Results of further investigations on this subject are recorded in this paper.

Methods of Analysis and Experimental Methods

These were in general similar to those described in Part I of this paper.

Role of carbohydrates in deamination by thermophilic bacteria

Stephenson and Gale (1937), Gale and Stephenson (1938) and Gale (1938) have found that *Bacteria coli* cells grown in nutrient broth containing glucose and chalk could not deaminate any of the aminoacids tested; whereas when growth medium was plain nutrient broth the cells could deaminate a large number of aminoacids. According to Lutwak-Mann (1936) *Bacteria coli* cells grown in tryptic broth containing 0·5% glucose could not deaminate or dephosphorylate adenosine triphosphate. Kendall (1922) found that presence of carbohydrates in growing cultures of bacteria prevents the formation of ammonia but it is not clear whether the formation of the deaminating enzyme system is checked or whether the apparent lack of production of free ammonia is due to the utilisation of free ammonia in any side reactions in the growing culture. The effect of carbohydrates on deamination by the thermophilic bacteria has been studied here in its two aspects, *i.e.*, firstly the effect of carbohydrates on the formation of the deaminating enzyme system and secondly their influence on the course of deamination when cells grown in plain nutrient broth were mixed with the aminoacid solutions.

The bacteria were cultivated simultaneously in the following three media:

(i) Nutrient broth.

(ii) Nutrient broth containing 2% dextrose and 1% calcium carbonate.

(iii) Nutrient broth containing 2% lævulose and 1% calcium carbonate.

Growth was more profuse in the media containing dextrose or lævulose as compared to the media without these carbohydrates. Further experimental procedure was as usual. The aminoacid used was *dl*-aspartic acid.

TABLE I

Organism	Growth medium	Mgm. dry wt. of the suspension per 5 ml.	Mgm. ammoniacal nitrogen liberated by 5 ml. of the suspension	Mgm. ammoniacal nitrogen liberated per 10 mgm. dry wt. of cells
B. thermophilus ..	Nutrient broth	12·01	0·522	0·435
	"	10·89	..	..
	Nutrient dextrose broth	10·78	0·483	0·448
	"	11·83	0·491	0·415
	Nutrient lævulose broth	13·05	0·557	0·427
	"	10·81	0·487	0·451
B. aerothermophilus	Nutrient broth	11·09	0·518	0·467
	"	10·88	..	..
	Nutrient dextrose broth	12·72	0·579	0·448
	"	12·65	0·576	0·455
	Nutrient lævulose broth	10·35	0·489	0·472
	"	13·17	0·630	0·478
B. thermoacidurans	Nutrient broth	13·63	0·612	0·449
	"	10·75	..	..
	Nutrient dextrose broth	12·52	0·545	0·435
	"	10·63	0·480	0·452
	Nutrient lævulose broth	11·78	0·534	0·453
	"	11·71	0·535	0·457

It is therefore obvious that the deaminating ability of these bacterial cells per mgm. dry weight is not at all depressed by the presence of dextrose or lævulose in the medium in which they were cultivated, in fact there is often an increase. It may be pointed out further that in the presence of the carbohydrates a far larger crop of the cells was obtained per 100 c.c. of the culture than when no carbohydrate was present in the medium. If ability to deaminate is compared on the basis of unit volume of culture or unit quantity nitrogen compounds present in the medium then the effect of dextrose and lævulose would seem to be to increase the deaminating ability of the cells considerably.

For studying the effect of carbohydrates on the course of deamination by cells grown in plain nutrient broth, dextrose or lævulose was added in a 2% concentration to a M/40 mixture of *dl*-aspartic acid and McIlvaine's

citrate-phosphate buffer. In the control experiments the addition of carbohydrates was omitted. Blank experiments were set up simultaneously both with and without dextrose and lævulose but omitting the aminoacid.

TABLE II

Organism	Remarks	Mgm. of ammonical nitrogen liberated per 5 ml. of cell suspension
B. thermophilus	No carbohydrate	0·575
	Do.	0·567
	2% Dextrose	0·513
	Do.	0·485
	2% Lævulose	0·623
	Do.	0·509
B. aerothermophilus	No carbohydrate	0·532
	Do.	0·539
	2% Dextrose	0·607
	Do.	0·522
	2% Lævulose	0·562
	Do.	0·478
B. thermoacidurans	No carbohydrate	0·505
	Do.	0·513
	2% Dextrose	0·575
	Do.	0·495
	2% Lævulose	0·483
	Do.	0·500

The effect of carbohydrates on the actual course of deamination is therefore irregular and inconsiderable, but there is no suppression of deamination.

Autodeamination of bacterial cells

It was noticed that cell suspensions of the organisms under study produced small but measurable amounts of ammonia even in the absence of any added aminoacids. To study this phenomenon in greater detail washed cell suspensions were prepared as usual. 10 ml. were added to 25 ml. of McIlvaine's citrate-phosphate buffer of appropriate pH and an aliquot portion was withdrawn immediately after shaking for analysis. The remainder of the mixture was incubated for thirty hours at 40° C. and analysed again for free ammonia. The data is summarised in Table III.

Autodeamination therefore takes place with all the three bacteria. The optimum pH is in the alkaline region; being 8·3 to 8·5 for *B. thermophilus* and *B. aerothermophilus* and about 8·0 for *B. thermoacidurans*.

TABLE III

Organism	pH	Mgm. of ammoniacal nitrogen liberated per 5 ml. of the suspension
B. thermophilus	5·5	0·013
	6·2	0·027
	6·9	0·041
	7·6	0·063
	8·3	0·093
	9·0	0·075
	9·7	0·037
B. aerothermophilus	5·5	0·017
	6·2	0·037
	6·9	0·059
	7·6	0·087
	8·3	0·112
	9·0	0·101
	9·7	0·065
B. thermoacidurans	5·5	0·025
	6·2	0·043
	6·9	0·061
	7·6	0·095
	8·3	0·107
	9·0	0·083
	9·7	0·053

Course of autodeamination at optimum pH

The previous experiments were repeated at optimum pH and with larger quantities of cell suspensions and buffer solutions. Samples were withdrawn at various intervals for analysis as shown below. The flasks were stoppered immediately after withdrawing a sample so as to prevent the loss of ammonia. The results are summarised in Table IV.

TABLE IV

Time after mixing Hours	Mgm. of ammoniacal nitrogen contained in by 5 ml. of suspension of washed cells		
	B. thermophilus	*B. aerothermophilus*	*B. thermoacidurans*
3 ..	0·000	0·000	0·000
6 ..	0·003	0·002	0·000
9 ..	0·010	0·007	0·000
13 ..	0·023	..	0·021
18 ..	0·046	0·041	0·051
24 ..	..	0·083	..
30 ..	0·109	0·141	0·121
36 ..	0·138	..	0·157
42 ..	0·147	0·203	..
48 ..	0·153	0·218	0·187

Very little autodeamination therefore takes place during the first few hours probably because very little aminoacids have been liberated through autolysis. As soon as appreciable autolysis has taken place deamination starts and proceeds more and more rapidly like an autocatalytic process till after thirty-six to forty-eight hours the rate decreases once again. Curves for rate of autodeamination have the S-shape typical of autocatalytic processes.

Effect of carbohydrates on autodeamination

5 ml. portions of the washed suspensions were mixed with 15 ml. of 2·5% dextrose or lævulose solutions prepared in buffer of pH equal to the optimum pH of autodeamination for each bacteria. In the control experiments no sugar was added to the buffer. Increase of ammonia was determined after incubation at 40° C. for twenty-four hours.

TABLE V

Organism	Carbohydrate	Increase in ammoniacal nitrogen, mgm./5 ml. of suspension
B. thermophilus	Nil	0·201
	Dextrose	0·088
	Lævulose	0·118
B. aerothermophilus	Nil	0·192
	Dextrose	0·108
	Lævulose	0·143
B. thermoacidurans	Nil	0·113
	Dextrose	0·064
	Lævulose	0·072

Thus both dextrose and lævulose suppress autodeamination.

Effect of storing suspensions on their deaminating ability

100 ml. of a washed suspension was prepared in each case and duplicate deamination experiments were carried out with aliquot portions from each suspension after every twenty-four hours for seven days. Each sample was withdrawn after shaking the suspensions well. The deaminating ability was tested against *dl*-aspartic acid at optimum pH.

Thus the deaminating capacity of cell suspensions of *Bacillus thermophilus* increases for three to four days and then decreases whereas that of *B. aerothermophilus* and *B. thermoacidurans* cell suspensions starts decreasing gradually from the very beginning.

TABLE VI

Age of Suspension Hours	Mgm. of ammoniacal nitrogen liberated by 5 ml. of the suspension		
	B. thermophilus	*B. aerothermophilus*	*B. thermoacidurans*
0 ..	0·563	0·586	0·621
24 ..	0·972	0·557	0·622
48 ..	1·432	..	0·601
72 ..	1·322	0·438	..
96 ..	..	..	0·465
120 ..	1·033	0·322	..
144 ..	..	..	..
168 ..	0·686	0·187	0·192

Effect of storing suspensions under hydrogen atmosphere

The experimental details regarding removal of oxygen and creation of as nearly perfect an hydrogen atmosphere as possible were the same as in case of deamination experiments under hydrogen, described in Part I of this paper. Each time a sample of the suspension was withdrawn for examination, the remainder of the suspension was re-evacuated and bubbled through with hydrogen. The aminoacid employed was M/40 *dl*-aspartic acid at optimum pH.

TABLE VII

Age of suspension. Hours	Mgm. of ammoniacal nitrogen liberated per 5 ml. suspension		
	B. thermophilus	*B. aerothermophilus*	*B. thermoacidurans*
0 ..	0·593	0·563	0·603
24 ..	1·457	0·561	0·611
48 ..	1·486	0·568	0·600
72 ..	..	..	..
96 ..	1·472	..	..
120 ..	1·441	0·542	0·587

Thus the decrease in deaminating ability noted in the previous experiment is not conspicuous when the storage is done under a reducing atmosphere. The decrease of activity of suspensions of *Bacillus aerothermophilus* and *B. thermoacidurans* is not very significant. The initial increase in activity of *B. thermophilus* suspensions is evident in this experiment also, but unlike storage under atmospheric conditions the subsequent decline in activity is not very noticeable here. Thus the destruction of deaminating power is an oxidative process.

Storing of suspensions and diffusion of the deaminating complex from the bacterial cells into the surrounding liquid

Suspensions were stored under hydrogen. After varying lengths of time samples were withdrawn and one-third of the sample was divided into two portions for estimation of the deaminating power of the whole suspension in duplicate. The remainder of the sample was centrifuged at 3,000 r.p.m. for ten minutes to give a sharp separation of a clear liquid and a sediment of cells. The clear liquid was carefully decanted off and its deaminating capacity determined. The sediment of cells was washed once in the centrifuge with a little distilled water and resuspended in distilled water so as to give the same volume as the volume of the suspension from which the cells were derived. The deaminating capacity of this suspension was also determined. In all cases the aminoacid employed was M/20 *dl*-aspartic acid at optimum pH.

TABLE VIII

Organism	Age of suspension	Mgm. ammoniacal nitrogen liberated by 5 ml. of whole suspension or its equivalent of cells or cell-free suspension		
		Whole suspension	Cell-free juice	Cells only
B. thermophilus ..	Fresh	0·621	0·008	0·622
	48 hours old	1·417	1·473	0·609
	96 ,,	1·625	1·886	0·600
B. aerothermophilus ..	Fresh	0·600	0·022	0·608
	48 hours old	0·589	0·134	0·601
	98 ,,	0·569	0·382	0·593
B. thermoacidurans ..	Fresh	0·584	0·043	0·571
	48 hours old	0·588	0·182	0·575
	98 ,,	0·573	0·349	0·569

The enzyme or enzyme complex responsible for deamination therefore diffuses out into the surrounding cell-free liquid during storage. A curious result is that when a forty-eight hour or ninety-six hour old suspension is separated into its cell portion and the cell-free juice, then the sum of the amount of ammonia liberated by these two fractions is much greater than that liberated by the original whole suspension or by the original whole suspension divided into two portions. In contrast to fresh whole suspensions whose deaminating properties are destroyed by narcotics and in contrast to all whole suspensions or cell portions thereof whose deaminating properties are suppressed by narcotics, such reagents have practically no effect on the deaminating power of the cell-free diffusion factor obtained by centrifuging

suspensions which have been stored for two to four days under hydrogen This will be obvious from Table IX.

Effect of narcotics on the deaminating power of cell-free diffusion factor

The effect of chloroform and toluene was examined.

TABLE IX

Organism	Treatment	Mgm. of ammoniacal nitrogen liberated per 5 ml. of whole suspension or the equivalent quantity of cells or cell-free juice			
		Fresh suspension	48 hours' old suspension	Cells from 48 hours' old suspension	Cell-free juice from 48 hours' old suspension
B. thermophilus ..	No treatment	0·578	1·431	1·401	0·867
	Chloroform	0·010	0·212	0·096	0·871
	Toluene	0·017	0·302	0·117	0·847
B. aerothermophilus	No treatment	0·744	0·737	0·712	0·363
	Chloroform	0·013	0·103	0·086	0·341
	Toluene	0·031	0·166	0·098	0·372
B. thermoacidurans	No treatment	0·663	0·671	0·643	0·296
	Chloroform	0·003	0·132	0·066	0·290
	Toluene	0·011	0·168	0·087	0·301

Relative thermostability of the endocellular and exocellular deaminating systems

The thermostability of the endocellular and exocellular deaminating enzyme systems was determined separately and the results summarised in the following table show that the exocellular enzyme is relatively more thermostable than the endocellular enzyme with all three bacteria. In all these experiments the portions of cell suspension or the cell-free centrifugates were kept at the stated temperature for five minutes and their deaminating power was then studied against *dl*-aspartic acid at optimum pH.

TABLE X

Temperature of heating °C.	Mgm of ammoniacal nitrogen liberated by cells or cell-free liquid equivalent to 5 ml. of suspension					
	B. thermophilus		*B. aerothermophilus*		*B. thermoacidurans*	
	Endocellular	Exocellular	Endocellular	Exocellular	Endocellular	Exocellular
40 ..	0·386	0·342	0·415	0·311	0·473	0·314
45 ..	0·389	0·346	0·423	0·318	0·479	0·304
50 ..	0·363	0·334	0·411	0·304	0·461	0·309
55 ..	0·311	0·341	0·414	0·322	0·471	0·318
60 ..	0·215	0·322	0·497	0·317	0·465	0·311
65 ..	0·068	0·295	0·343	0·309	0·423	0·306
70 ..	..	0·161	0·041	0·301	0·036	0·295
75 ..	..	0·008	..	0·213	..	0·201

Discussion and Summary

The thermophilic bacteria *Bacillus thermophilus, B. aerothermophilus* and *B. thermoacidurans* oxidatively deaminate aminoacids. Cells grown in carbohydrate broth are as efficient deaminating agents as those grown in plain broth. In fact as the growth is much more profuse in presence of carbohydrates like dextrose and lævulose the total quantity of deaminating enzyme system formed in a given volume of the growth medium is greater in presence of the carbohydrates than in their absence. Presence of carbohydrates during actual deamination in mixtures of washed cell suspensions and aminoacid solutions does not have any significant effect. In their behaviour towards carbohydrates therefore the thermophilic bacteria and their deaminating enzymes behave in a manner different from that of other bacteria studied by previous investigators. Apart from deaminating added aminoacids the cells can deaminate the aminoacids produced by their own autolysis. The time curve of this process possesses the S-shape typical of autocatalytic processes. In the beginning when only small amounts of aminoacids have been produced the rate of ammonia formation is small; as autolysis results in the formation of more and more aminoacids the rate of ammonia production also increases rapidly till after thirty-six or forty-eight hours the rate decreases once again. When dextrose or lævulose is added to the cell suspensions undergoing autolysis and autodeamination then the amount of ammonia formed is decreased; in this respect therefore autodeamination differs from deamination of added aminoacids.

The deaminating capacity of cell suspensions of *Bacillus aerothermophilus* and *B. thermoacidurans* gradually decreases on storage, whereas that of *B. thermophilus* cell suspensions increases at first and then decreases. This decrease of deaminating ability is considerably reduced if the cell suspensions are stored in the absence of oxygen. Destruction of the deaminating enzyme system is therefore an oxidative process. Cell-free centrifugates obtained from stored cell suspensions go on increasing in deaminating ability with the increase in age of the suspension. The residual cells are still capable of deamination. When a two or three days old suspension is seperated into its cell portion and the cell-free liquid then the sum of the amount of ammonia liberated separately by these two portions is greater than that produced by the original whole suspension or by the original whole suspension divided into two parts. Chloroform and toluene destroy the deaminating activity of fresh cell suspensions, that of stored suspensions is only partially suppressed, whereas cell-free centrifugates of stored suspensions are not affected by chloroform or toluene. The thermostability of the deaminating enzyme in the cell-free liquid is greater than that of the

deaminating enzyme in the cells. It would therefore appear that with all the three bacteria under study there exist two distinct deaminating enzymes. One of these is endocellular and is intimately connected with the life-process of the cells and is therefore rendered ineffective when the cells are treated with toluene or chloroform, or are warmed to their thermal death point. The second deaminating enzyme system is exocellular, and is formed when cell suspensions are stored at room temperature especially under hydrogen. This exocellular factor is not affected by toluene or chloroform and is relatively more thermostable than the endocellular deaminating enzyme system. The endocellular enzyme is gradually destroyed when the suspensions are stored under atmospheric conditions. The exocellular deaminating enzyme system is much more stable against oxidative destruction than the endocellular deaminating enzyme. When both are present together, as for example in whole suspensions which are two or three or more days' old, then they partially and reversibly neutralise each other since the amount of ammonia produced by the two factors separately is much greater than when they are both present together.

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CARBOHYDRATES AND MICROBIAL PROTEINASES

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Received July 5, 1945

The question whether carbohydrates affect proteinase formation by micro-organisms is of interest in soil and in agricultural biochemistry. It is generally believed that presence of carbohydrates suppresses proteolytic activity and proteinase formation by micro-organisms. This inhibitive effect is usually explained by assuming that carbohydrates are preferentially utilised by the growing cells as their source of energy and carbon and that in their presence the micro-organisms attack proteins or peptone solely for obtaining the nitrogen with which to build their protoplasm proteins. The enzymic system of the cells being adaptive in nature the amount of protein decomposing enzyme formed is therefore considerably reduced. See, for example, Kendall (1922). Console and Rahn (1938) have shown that filtrates from cultures of *Bacillus subtilis* were proteolytically inactive when glucose was present in the growth medium. According to Wilson (1930), however, in case of *B. pyocyaneus*, proteinase formation was not affected by the presence of carbohydrates in the growth medium.

The experimental technique adopted by most previous investigators to study the effect of carbohydrates on proteinase formation seems to have been to grow the micro-organism in media containing the carbohydrates and to test the culture filtrates for proteolytic activity. The present investigations were carried out with the soil aerobe *Bacillus cereus* and with the typical thermophilic bacteria *B. thermophilus*, *B. aerothermophilus* and *B. thermoacidurans* and the conclusions are here published in view of their somewhat divergent nature from those of earlier investigators. The bacteria selected for the present study are all fairly widespread in soil, etc., and are responsible for a large number of enzymic transformations which are of importance in soil and agricultural biochemistry and in most thermophilic fermentations.

The organisms were grown in nutrient broth containing the respective carbohydrate in a 2% final concentration. The cultures were incubated for forty-eight hours at 50° C. for the thermophilic bacteria and at 37° C. for *Bacillus cereus* after which they were centrifuged and filtered through chamberland candles yielding cell-free filtrates whose proteolytic activity was tested on gelatin solutions by following the rapid decrease

in their viscosity and by measuring the increase in free α-amino acids by the micro Van Slyke method. In the viscometric method the initial velocity of hydrolysis was calculated by extrapolating viscosity/time curves to zero time and also the per cent. fall in initial viscosity in thirty minutes was calculated from the same set of observations.

TABLE I

Effect of Carbohydrates

Carbohydrate in 2% final concentration	Initial velocity of hydrolysis, % fall in initial viscosity in first 5 minutes	% Fall in initial viscocity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
	Bacillus cereus		
Nil ..	7·76	54·65	7·01
Dextrose ..	0·81	4·95	0·66
Lævulose ..	0·32	2·60	0·00
Sucrose ..	4·11	29·45	3·87
Glycerol ..	7·05	48·60	6·73
Dextrin ..	7·41	50·30	6·83
Starch ..	7·83	53·90	7·09
	Bacillus thermophilus		
Nil ..	6·79	49·85	6·13
Dextrose ..	0·41	4·15	0·73
Lævulose ..	0·17	1·75	0·89
Sucrose ..	3·23	26·10	2·93
Glycerol ..	5·87	40·05	5·07
Dextrin ..	6·48	47·95	5·79
Starch ..	6·77	50·15	6·03
	Bacillus aerothermophillus		
Nil ..	7·35	52·45	6·49
Dextrose ..	0·63	5·20	0·72
Lævulose ..	0·19	6·05	0·00
Sucrose ..	2·97	20·10	2·13
Glycerol ..	5·83	39·75	5·07
Dextrin ..	6·12	43·15	5·31
Starch ..	6·89	48·60	6·12
	Bacillus thermoacidurans		
Nil ..	7·07	50·10	6·31
Dextrose ..	0·35	3·20	0·71
Lævulose ..	0·32	3·05	0·79
Sucrose ..	2·31	18·10	1·82
Glycerol ..	5·82	38·75	5·13
Dextrin ..	6·47	47·30	5·68
Starch ..	6·88	49·95	6·20

The above results show that the presence of carbohydrates in the growth medium results in a decrease of the proteinase activity of cell-free filtrates. The effectiveness of the carbohydrates in depressing proteinase activity of the filtrates is in the following increasing order: starch, dextrin, glycerol, sucrose, dextrose and lævulose and this is also the increasing order of availability of these carbohydrates for attack and assimilation by the bacteria. The bacterial growth in dextrose, lævulose and in sucrose broths was much

more profuse than in plain broth, yet cell-free filtrates from the cultures in the former media showed negligible proteolytic activity.

TABLE II

Effect of dextrose at different concentrations

Carbohydrate		Mgm. dry weight of cells per 100 ml. of culture	Initial velocity, % fall in initial viscosity in first 5 mintues	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
	%	*Bacillus cereus*		
Nil	..	50·5	7·32	6·41
Dextrose	0·10 ..	68·0	7·48	6·57
,,	0·25 ..	115·7	7·36	6·89
,,	0·50 ..	149·0	7·95	7·01
,,	0·75 ..	173·5	6·30	5·45
,,	1·00 ..	188·3	3·15	2·72
,,	1·50 ..	196·5	1·52	1·17
,,	2·00 ..	200·4	0·63	0·89
		Bacillus thermophilus		
Nil	..	15·3	6·37	5·41
Dextrose	0·10 ..	30·0	6·52	5·57
,,	0·25 ..	46·7	7·01	5·89
,,	0·50 ..	69·3	7·00	5·95
,,	0·75 ..	80·2	5·43	4·61
,,	1·00 ..	92·4	2·15	1·58
,,	1·50 ..	100·0	1·03	0·95
,,	2·00 ..	106·5	0·54	0·60
		Bacillus aerothermophilus		
Nil	..	43·0	6·15	5·27
Dextrose	0·10 ..	68·5	6·38	5·36
,,	0·25 ..	89·6	6·75	5·83
,,	0·50 ..	117·5	6·89	5·96
,,	0·75 ..	142·3	6·01	5·02
,,	1·00 ..	166·2	3·82	3·17
,,	1·50 ..	183·4	2·41	1·78
,,	2·00 ..	190·0	0·95	0·88
		Bacillus thermoacidurans		
Nil	..	48·1	6·37	5·49
Dextrose	0·10 ..	60·4	6·55	5·56
,,	0·25 ..	90·2	6·82	5·88
,,	0·50 ..	120·0	6·70	6·02
,,	0·75 ..	139·5	6·01	5·00
,,	1·00 ..	150·8	3·15	2·32
,,	1·50 ..	170·5	1·68	0·95
,,	2·00 ..	190·9	0·85	0·43

As is evident from the above tables dextrose and lævulose upto about 0·5% concentration actually increase the proteinase content in the culture filtrates, probably because of increased cell formation. Beyond this concentration the effect of increased cell formation is masked by the inhibitive effect of the sugars on proteinase activity of the filtrates, till when the concentration of sugars reaches 2% the filtrates have negligible proteolytic activity. The amount of bacterial mass produced in each case was measured

TABLE III

Effect of lævulose at different concentrations

Carbohydrate	%	Mgm. dry weight of cells per 100 ml. of culture	Initial velocity, % fall in initial viscocity in first 5 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
		Bacillus cereus		
Nil	..	50·5	7·32	6·41
Lævulose	0·10 ..	85·0	7·68	6·80
,,	0·25 ..	130·2	7·89	7·02
,,	0·50 ..	165·5	7·80	6·89
,,	0·75 ..	180·4	6·16	5·83
,,	1·00 ..	193·0	3·20	2·24
,,	1·50 ..	205·6	1·23	1·85
,,	2·00 ..	210·4	0·32	0·00
		Bacillus thermophilus		
Nil	..	15·3	6·37	5·41
Lævulose	0·10 ..	35·6	6·49	5·53
,,	0·25 ..	65·2	6·75	6·02
,,	0·50 ..	80·3	6·60	6·01
,,	0·75 ..	90·5	3·85	2·95
,,	1·00 ..	100·4	2·24	1·68
,,	1·50 ..	115·4	1·11	0·95
,,	2·00 ..	120·0	0·38	0·05
		Bacillus aerothermophilus		
Nil	..	48·1	6·37	5·49
Lævulose	0·10 ..	100·3	6·63	5·72
,,	0·25 ..	130·2	6·95	6·15
,,	0·50 ..	135·8	7·13	6·20
,,	0·75 ..	150·5	5·33	5·00
,,	1·00 ..	170·5	4·38	3·85
,,	1·50 ..	183·2	2·35	1·82
,,	2·00 ..	198·6	0·36	0·08
		Bacillus thermoacidurans		
Nil	..	43·0	6·15	5·27
Lævulose	0·10 ..	..	6·48	..
,,	0·25 ..	..	6·92	6·10
,,	0·50 ..	120·4	6·95	6·05
,,	0·75 ..	150·6	5·63	..
,,	1·00 ..	..	..	..
,,	1·50 ..	..	..	..
,,	2·00 ..	206·4	0·27	0·13

by filtering cultures through the filtration tube of a previously weighed microbeaker and re-weighing after washing the bacterial mass and drying over phosphorus pentoxide in vacuum at room temperature.

The above findings confirm the opinions of the earlier workers, namely that carbohydrates restrict proteinase formation. But the question now arises whether the effect of carbohydrates is actually that of prevention of proteinase formation, or whether they simply prevent the diffusion of the proteinase from the dead or living cells into the surrounding medium or whether presence of carbohydrates in the growth medium renders the proteinase formed incapable of functioning in the absence of the living cells.

Experiments were therefore conducted to determine the proteinase content of whole cultures, cells alone and cell-free filtrates, when say lævulose was present in the medium at start. For determining the proteinase content of cells the latter were reaped by centrifuging and were re-suspended in water to give the same volume as that of the culture they were obtained from. In every case 2 ml. of the whole culture, cell suspension or cell-free filtrate were mixed with 10 ml. of the gelatin substrate and viscometric observations and Van Slyke α-amino nitrogen estimations were carried out as usual.

TABLE IV

Cultures in media containing 2% lævulose

Organism	Source of proteinase	Initial velocity, % fall in initial viscosity in first 5 minutes	Increase in α-amino nitrogen, mgm./10 m. in 48 hours
Bacillus cereus ..	Whole culture	6·38	5·49
	Cell-free filtrate	0·41	0·08
	Cells alone	6·01	5·32
Bacillus thermophilus ..	Whole culture	5·68	4·87
	Cell-free filtrate	0·22	0·00
	Cells alone	5·11	4·32
Bacillus aerothermophilus ..	Whole culture	6·27	5·43
	Cell-free filtrate	0·11	0·03
	Cells alone	6·01	5·29
Bacillus thermoacidurans ..	Whole culture	6·13	5·33
	Cell-free filtrate	0·29	0·11
	Cells alone	5·75	5·13

TABLE V

Cultures in media containing 2% dextrose

Organism	Source of proteinase	Initial velocity, % fall in initial viscosity in first 5 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
Bacillus cereus ..	Whole culture	5·82	5·32
	Cell-free filtrate	0·02	0·00
	Cells alone	5·75	5·22
Bacillus thermophilus ..	Whole culture	5·93	5·21
	Cell-free filtrate	0·18	0·15
	Cells alone	5·80	5·00
Bacillus aerothermophilus ..	Whole culture	6·50	6·12
	Cell-free filtrate	0·32	0·27
	Cells alone	6·29	5·39
Bacillus thermoacidurans ..	Whole culture	6·01	5·35
	Cell-free filtrate	0·20	0·05
	Cells alone	5·65	5·02

It is obvious therefore that dextrose and lævulose do not actually prevent proteinase formation but that the proteinase produced either does

not pass out into the cell-free medium or that it does not function in the absence of living cells.

Attempts were then made to see if any process could be devised to make the proteinase diffuse out of the cells. Autolysing the cells in various ways failed to achieve the purpose. Autolysis merely resulted in destruction of the proteinase within the cells as is obvious from Table VI.

TABLE VI

Treatment	*Bacillus cereus*, % fall in initial viscosity in first 5 minute	*Bacillus cereus*, increase in α-amino N. mgm./10 ml. in 48 hours	*B. thermophilus*, % fall in initial viscosity in first 5 minutes	*B. thermophilus*, increase in α-amino N. mgm./10 ml. in 48 hours	*B. aerothermophilus*, % fall in initial viscosity in first 5 minutes	*B. aerothermophilus*, increase in α-amino N. mgm./10 ml. in 48 hours	*B. thermoaciduranus*, % fall in initial viscosity in first 5 minutes	*B. thermoaciduranus*, increase in α-amino N. mgm./10 ml. in 48 hours
1. Suspensions of cells, untreated	7·32	6·48	6·69	5·86	7·11	5·93	6·95	6·10
2. Autolysed with toluene for 8 hours at 40° C., autolysate	0·05	0·00	0·00	0·00	0·00	0·00	0·00	0·00
3. Same as 2, cells ..	1·31	0·93	0·86	0·52	0·43	0·00	1·83	1·01
4. Autolysed with chloroform for 4 hours at 40° C., autolysate	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
5. Same as 4, cells ..	2·41	1·43	1·42	0·83	1·86	1·01	0·93	0·05
6. Glycerol-phosphate, 3 hours at room temp., autolysate	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
7. Same as 6, cells ..	3·93	3·04	4·15	3·29	3·00	2·17	3·41	2·13
8. Autolysed with toluene, 4 hours at 20° C., autolysate	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
9. Same as 8, cells ..	1·60	1·32	2·31	1·05	1·43	0·62	0·71	0·00
10. Phenole — gelatine, 6 hours at 37° C., autolysate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
11. Same as 10, cells ..	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

It therefore seems that killing of cultures and autolysis results in destruction of the proteolytic activity of these cultures. For the sake of comparison these experiments were repeated with cultures grown in plain nutrient broth, *i.e.*, in the absence of any carbohydrate and the results summarised in Table VII show that destruction of the cell life process in carbohydrate-free cultures does not result in destruction of the proteolytic activity. Autolysis of these carbohydrate-free cultures was always carried out at 40° C. under toluene for eight hours.

TABLE VII

Organism	Culture	Initial velocity of hydrolysis, % fall in initial viscosity in first 5 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
Bacillus cereus ..	Alive	7·32	6·58
	Autolysed	7·18	6·53
B. thermophilus ..	Alive	6·88	6·07
	Autolysed	6·81	6·00
B. aerothermophilus ..	Alive	6·92	6·17
	Autolysed	7·01	6·13
B. thermoacidurans ..	Alive	6·13	5·32
	Autolysed	6·10	5·37

DISCUSSION AND SUMMARY

With the four bacteria *Bacillus cereus, B. thermophilus, B. aerothermophilus* and *B. thermoacidurans* here examined, presence of carbo-hydrates in th growth media results in a decrease in the proteolytic activity of the culture filtrates. The depressing effect of the carbohydrates is proportional to the degree of availability of the carbohydrate for attack by the bacteria. Although the proteolytic activity of the culture filtrates is descreased proteinase formation is not prevented or depressed by the presence of carbohydrates in the growth medium. The cells reaped from cultures prepared in carbohydrate media are very rich in proteinase but it is very difficult to extract the proteinase from the cells. The effect of carbohydrates is thus to make the proteinase intimately combined with some cell constituents and is not to prevent or hinder the formation of proteinase.

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898.48—Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent
and Published by The Indian Academy of Sciences, Bangalore

ON THE CELL-DIVISION AND MITOSIS IN SOME SOUTH INDIAN DIATOMS*

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Received September 24, 1945

(Communicated by Prof. M. O. P. Iyengar)

(With 88 Text-Figures and 1 Plate)

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Introduction

Lauterborn (1896) was the first to give a detailed account of mitosis in Diatoms. He gave an account of the nuclear division in five Diatoms, *viz.*, *Surirella Capronii* (= *S. calcarata*), *Navicula oblonga* (= *Pinnularia oblonga*), *Pinnularia viridis*, *Nitzschia sigmoidea* and *Gyrosigma attenuatum* (= *Pleurosigma attenuatum*). Other authors following him studied mitotic division in the following forms:—*Eunotia major* (Wisselingh, 1913), *Coscinodiscus subbuliens* (Ikari, 1923), *Achnanthidium brevipes* (Gemeinhardt, 1925), *Synedra ulna*, *S. pulchella* and *S. affinis* (Gemeinhardt, 1926), *Navicula peregrina* (Kolbe, 1927), *Cocconeis placentula* (Geitler, 1927), *Rhoicosphenia curvata* (Cholnoky, 1927 *a*), *Diatoma vulgare* (Cholnoky, 1927 *b*), *Navicula radiosa* and *Eunotia* '*formica*' (Geitler, 1929), *Melosira granulata* (Kreiger, 1927), *Coscinodiscus biconicus* (Hofker, 1930), *Meredion circulare* (Geitler, 1932), *Biddulphia sinensis* (Schmidt, 1927, 1933), *Melosira arenaria* (Cholnoky, 1933), *Ditylum Brightwellii* (Gross, 1937/38), *Cyclotella Meneghiniana* (Iyengar and Subrahmanyan, 1944) and *Navicula halophila* (Subrahmanyan unpublished). In most of these cases all the details of the mitosis were not fully followed. Only a few stages were recorded in *Eunotia major*, *Synedra*

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Part of the Thesis approved for the Degree of Master of Science of the University of Madras.

pulchella, S. affinis, Navicula peregrina, Rhoicosphenia curvata, Meredion circulare and *Ditylum Brightwellii*; and, in *Achnanthidium brevipes, Synedra ulna, Eunotia formica* and *Melosira granulata* a few more stages have been recorded, but all the stages were not fully observed. Only in four cases were all the stages of the mitosis worked out so far, *viz.*, (1) *Surirella Capronii*, (2) *Melosira arenaria*, (3) *Cyclotella Meneghiniana* and (4) *Navicula halophila*.

Practically no work has been done on the cytology of any Diatom in India so far. At the kind suggestion of Professor M. O. P. Iyengar, the writer took up five Diatoms (three belonging to the Centrales and two to the Pennales), which occur commonly in Madras, for a detailed study of their mitosis and cell-division.

1. *Terpsinoë musica* Ehrenberg

This Diatom was found growing as an epiphyte on *Cladophora* and *Pithophora* in a pond inside the Agri-Horticultural Gardens at Madras. The Diatom grows in long zig-zag chains formed by the cells being held together by means of small mucilage pads at the corners (Pl. XXIX, Figs. 2 and 4).

Living material of the *Cladophora* and *Pithophora* with the Diatom growing on them was collected and kept in the laboratory in glass troughs containing the pond water. The Diatom was fixed throughout the twenty-four hours of the day at intervals of one hour each. As it was found difficult to separate the Diatom from the host alga, it was fixed along with the latter. Division generally took place in the evenings between 4 and 6 P.M.

The following fixing fluids were employed:—Chrom-acetic, Flemming's weak solution, Flemming-Benda, Schaudinn's sublimate—acetic alcohol Allen's Bouin (PFA_3), Zenker's fluid, Hofker's fluid, Gilson-Petrunkewitsch and vom Rath's fluid (without platinic chloride). The Diatom was fairly well fixed in all these fluids, but PFA_3 and hot Schaudinn's fluid proved the most satisfactory. Of these two latter fluids, PFA_3 was slightly better than Schaudinn's.

Since after fixation the Diatom chains break up into the individual cells and also break away from the host alga, bits of the host filaments were frequently shaken in the fixing fluid, and later on in the washing fluids also, to free them of the Diatoms, which then fell to the bottom and got accumulated there. The accumulated Diatom sediment was generally mixed with a lot of dirt. In order to separate the Diatom from the dirt, the sediment was transferred to clear washing fluid in a petri-dish and the dish was gently rocked to and fro and then held somewhat slantingly. After about a couple

of minutes the dirt generally accumulated in the deeper portion of the liquid while the Diatom cells gathered together near the shallow side of the liquid in the dish from where they were carefully removed with the help of a fine pipette and transferred into a tube containing fresh washing fluid. By repeating this process several times, most of the cells could be separated.

The material separated in this manner was washed thoroughly in water or 50% alcohol (according to the fixing fluid employed) by the decantation method and after passing through the necessary grades was finally preserved in 70% alcohol.

The following stains were employed:—Heidenhain's iron-alum hæmatoxylin, safranin, gentian violet, Mayer's hæmalum, Newton's gentian violet, safranin-light green and safranin-gentian violet. Iron-alum hæmatoxylin proved to be the most satisfactory. Excellent preparations were also obtained by staining the material in Feulgen's stain.

Smear preparations of the material were made according to protozoological methods (McClung, 1937, pp. 530–31). The material was also stained *in toto*. The following schedule was followed for smear preparations. A small quantity of the material in 70% alcohol was spread on slides previously smeared with a thin coating of Mayer's albumen, and, before the alcohol dried up completely, the slides were placed in 85% alcohol and left there for about 5 minutes. The slides were then brought down the alcohol grades into water and then washed thoroughly, mordanted in 4% iron-alum solution for 1½ hours, washed in running water for 15 minutes and then stained in ½% hæmatoxylin for about 4 hours. The staining was differentiated in saturated solution of picric acid. After thorough washing for 3 to 4 hours the slides were passed up through the alcohol grades, cleared in xylol and finally mounted in Canada balsam. Better preparations were obtained when the material was bleached in 10% hydrogen peroxide in 30% alcohol prior to mordanting. Material fixed in osmic fixatives (*e.g.*, Flemming's weak formula and Flemming-Benda) while being bleached was placed in the sun for thorough bleaching.

For staining *in toto*, the material from 70% alcohol was brought down to water and washed well, the changes from one liquid to another being made by decantation. The schedule mentioned above was followed for staining the material. After dehydrating and clearing, the material was transferred to dilute balsam for infiltration and slides were prepared when the balsam reached the desired consistency.

Aceto-carmine preparations also were frequently made for observing division figures. The material stained in aceto-carmine was made permanent as per method given by Lee (1937, p. 687).

Microtome sections of the Diatom were made after embedding the fixed material in paraffin. Sections were cut 20 μ thick. Some excellent views of prophase stages were obtained in these sections, especially valve views, which are otherwise very difficult to obtain owing to the thin and flat nature of the Diatom cell.

Vegetative cell.—The cells of the diatom are rectangular to square with rounded corners in girdle view (Text-Fig. 2) and linear-elliptical with undulations in valve view (Text-Fig. 1). In valve view the poles are capitate. The valves possess transapical septa, usually four in number; but, cells possessing six septa and small cells having only two septa were also frequently met with. The cytoplasm forms a lining layer and a large nucleus is suspended by cytoplasmic strands at the centre of the cell. Numerous disc-shaped or somewhat dumb-bell shaped golden yellow chromatophores are distributed throughout the cell more or less uniformly (Text-Fig. 2).

Mitosis.—The resting nucleus is usually spherical but often appears compressed. The nucleus shows one, occasionally two or three nucleoli and a well-stained reticulum. The chromatin granules stand out very clearly in the reticulum (Text-Fig. 3).

At the beginning of prophase, thin chromosomal threads are seen in the nucleus (Text-Fig. 4). The individual chromosomes next become clearly distinguishable (Text-Figs. 5 and 6). In the meanwhile the cell increases somewhat in volume, the valves loosening a little to allow for this increase. The nucleus still remains at the centre of the slightly enlarged cell. There is no movement of the nucleus in this Diatom towards one side as observed by Cholnoky (1933 *b*) in *Melosira arenaria* and by the writer in *Navicula halophila* (Subrahmanyan, unpublished).

In late prophase a large number of long thread-like chromosomes is seen distributed in the nuclear cavity (Text-Fig. 7; Pl. XXIX, Fig. 9). In the valve view a few chromosomes showed a slightly constricted portion, which was somewhat more lightly stained than the remaining portion of the chromosome. This probably represents the spindle attachment region.

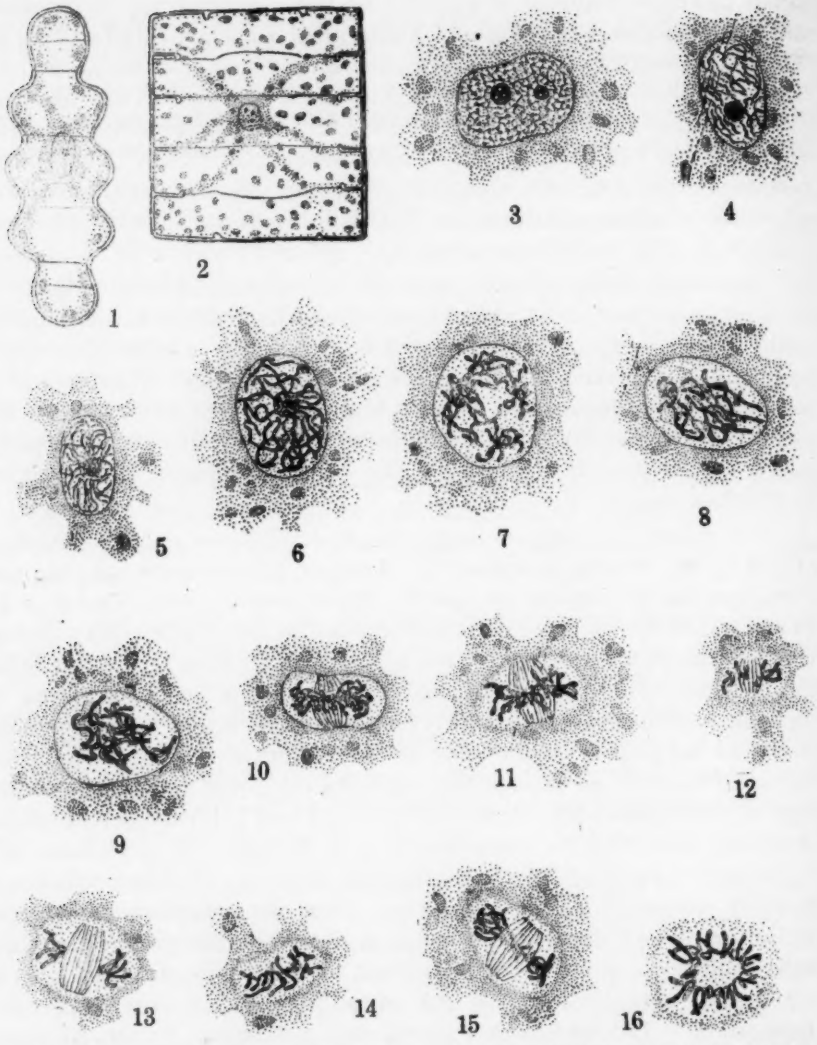
In late prohase there is no trace of the nucleolus (Text-Fig. 7). The nuclear membrane persists for a long time; in some instances it was seen even during metaphase (Text-Fig. 10). And even after the disappearance of the nuclear membrane the nuclear space is seen standing out clearly (Text-Fig. 11).

In early metaphase the chromosomes are seen directed towards one side of the nuclear cavity all around the spindle which becomes first evident

about this time (Text-Figs. 8 and 9). At late metaphase the chromosomes are seen arranged more or less in a ring around the spindle (Text-Fig. 16). The distribution of the chromosomes in the equatorial plane is not uniform. There are more chromosomes in one half of the ring than in the other, and a small gap could be seen in that half where the chromosomes are fewer (Text-Figs. 12–14). The spindle is barrel-shaped (Pl. XXIX, Fig. 7). No centrosome could be seen, though in a few preparations a few granules were observedat the poles of the spindle (Text-Fig. 15), as was found by Cholnoky (1933 *b*, p. 708) in *Melosira arenaria*.

The chromosome number could not be determined with certainty as no clear polar view of metaphase was obtained. Further, as the chromosomes are long and differently shaped and somewhat compactly arranged, individual chromosomes could not be recognised clearly. However, in an aceto-carmine preparation, a slighly oblique polar view of metaphase was obtained (Text-Fig. 16) in which the chromosome number could be approximately estimated as about 28 (2 *n*). This number is supported by the counts in prophase stages.

The chromosomes appear longitudinally divided even in early metaphase (Text-Fig. 9). During anaphase the daughter chromosomes separate and move towards the poles of the spindle. The anaphasic figures (Text-Figs. 17, 18 and 20; Pl. XXIX, Fig. 8) are characterised by long trailing chromosomes which lie parallel to the axis of the spindle and in some cases the sister chromosomes could still be distinguished for quite a long time owing to their incomplete separation. Very early anaphase stages were not found in any of the preparations in spite of careful search of numerous preparations. Therefore, an attempt was made by the writer to follow with the help of water immersion objectives (N.A. ·75 and 1·18) the several stages of nuclear division in the living specimens to find out if by any chance any of the earlier stages of anaphase could be observed. Dividing cells could be easily recognised by the aggregation of the chromatophores around the nuclear cavity and also by the orientation of the chromatophores along the plane of division. At this stage, the nucleus, when carefully examined, showed a prophasic condition with the long thread-like chromosomes clearly distinguishable. The nucleus then passed into metaphase. In this stage the spindle is barrel-shaped and the chromosomes are seen arranged in a ring around the equator of the spindle. After late metaphase, the two sets of daughter chromosomes were observed to move away from each other very rapidly as if pulled apart by some force and finally come to lie at the poles of the spindle. This anaphasic movement was observed in dozens of individuals and in every case was found to be extremely rapid, the movement



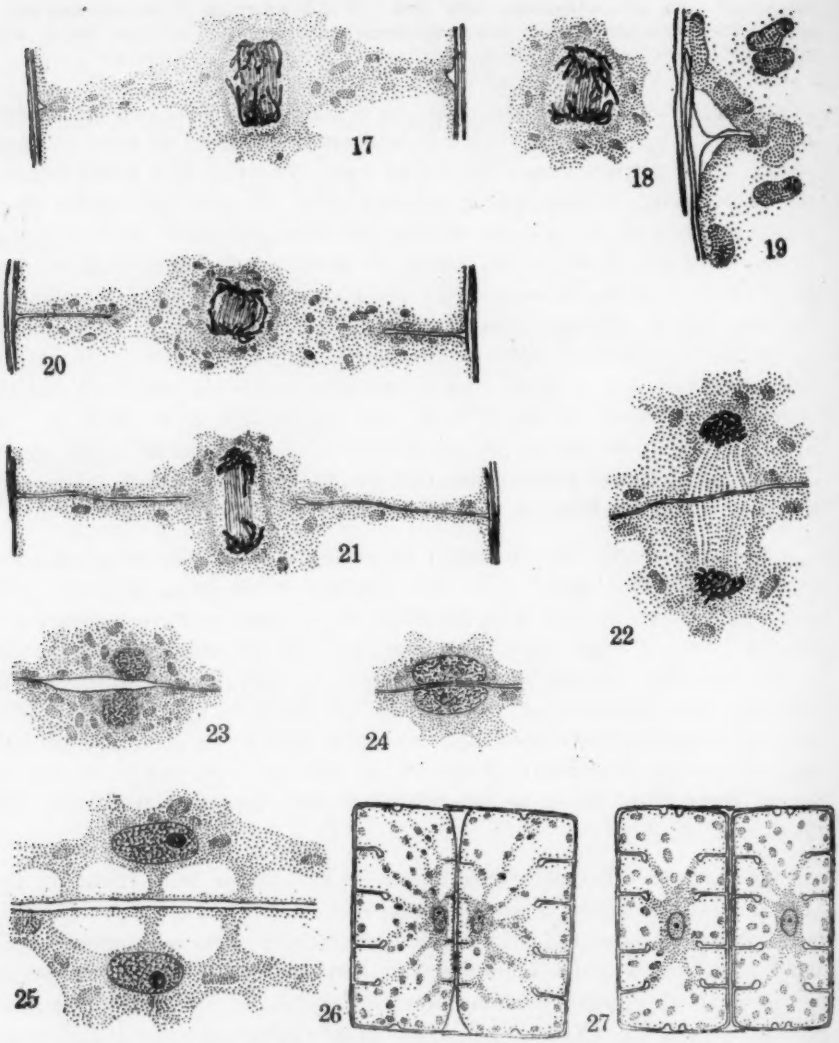
Text-Figs. 1–16. *Terpsinoë musica*.—Fig. 1. Cell showing nucleus and chromatophores (valve view). Fig. 2. Do. (girdle view). Fig. 3. Resting nucleus. Note vacuolate nucleoli. Figs. 4–5. Early prophase. Fig. 6. Prophase. Nucleolus still present. Fig. 7. Late prophase, seen from valve side; nucleolous disappeared. Figs. 8–9. Early metaphase. Fig. 10. Metaphase; nuclear membrane still intact. Fig. 11. Late metaphase; nuclear membrane disappeared. Figs. 12–14. Metaphase in three different foci, showing the arrangement of the chromosomes around the spindle. Fig. 12, upper focus; Fig. 13, median focus; Fig. 14, lower focus. Fig. 15. Metaphase, median focus showing tiny granules at the poles of

the spindle. Fig. 16. Metaphase, polar view. Note arrangement of the chromosomes in a ring; the spindle fibres seen as dots in the centre of the ring. Fig. 1, ×400; Fig. 2, ×275; rest, ×880.

taking hardly 4 to 5 seconds. This rapid separation of the daughter chromosomes during anaphase evidently explains the absence of early anaphase stages in the preparations. The writer was, however, able to fix some of the earlier stages of anaphase by keeping some cells on a slide under observation and quickly fixing it by running acetic-alcohol under the cover galss as the daughter chromosomes began to move apart. Aceto-carmine preparations were made of these stages. The stage shown in Fig. 17 was obtained by this method. It may be mentioned in this connection that, while investigating the life-history of *Ditylum Brightwellii*, Gross (1937/38, p. 7) was not able to observe even a single case of mid-anaphase. He presumed that the anaphasic movement in the Diatom took place with great rapidity. The writer's observations on the present Diatom (*Terpsinoë musica*) would appear to fully justify Gross' presumption that the anaphasic movement takes place very rapidly in his Diatom.

The spindle increases in length progessively as the daughter chromosomes move farther apart. This was observed in the living material. The chromosomes having moved as far apart as possible, begin to contract and show a clumped appearance (Text-Figs. 21 and 22) and soon the outline of the individual chromosomes becomes very difficult to distinguish. In telophase, the two groups of chromosomes are at first far apart (Text-Fig. 22), but after cytokinesis is complete the two nuclei come to lie very close to each other (Text-Figs. 23 and 24). Later on, as the new valves are being formed the daughter nuclei move to the centre of the respective daughter cells (Text-Figs. 25–27).

Cytokinesis.—Though several stages of cytokinesis were found in the fixed material, the actual details of cytokinesis as observed in the living specimens were most interesting. In the first place the process is extremely rapid and is completed within 3–4 minutes. Simultaneously with the rapid anaphasic movement of the daughter chromosomes, a cleavage furrow starts at the periphery of the cell (Pl. XXIX, Fig. 3) and gradually advances towards the centre of the cell. In optical section (in girdle view) it is seen as a small cleavage furrow starting just at the middle where the two valves overlap each other (Text-Figs. 17 and 19; Pl. XXIX, Fig. 6). The cleavage further advances inwards very rapidly until it reaches the spindle region of the division figure (Text-Fig. 21). But, after reaching the spindle region, the cleevage process becomes very much slowed up as if some kind of resistance is being offered by the spindle. Finally



Text-Figs. 17–27. *Terpsinoë musica*.—Fig. 17. Early anaphase. Note beginning of cytokinesis from two girdle sides (optical section); aceto-carmine preparation. Fig. 18. Anaphase. Fig. 19. Beginning of cytokinesis; from a specimen just killed with osmic acid. Fig. 20. Anaphase; cytokinesis advanced half way to the centre. Fig. 21. Late anaphase; cytokinesis nearing completion; aceto-carmine preparation. Fig. 22. Telophase; cytokinesis just completed; aceto-carmine preparation. Fig. 23. The two nuclei which were very much apart during late telophase (Fig. 22) have come very close to each other at this stage. Fig. 24. Daughter nuclei fully formed. Nucleolus lightly stained. Fig. 25. Daughter nuclei moving

towards the centre of their respective cells. Fig. 26. The two daughter cells. New transverse septa being formed at the centre. Note cytoplasmic strands connecting them with the nucleus. Fig. 27. The two daughter cells after formation of valves and transverse septa. Figs. 17, 20 and 21, ×575; Figs. 18, 22–25, ×890; Fig. 19, ×1170; Figs. 26 and 27, ×250.

the cleavage cuts through the spindle and the cytokinesis becomes complete. The whole process from the commencement of the cleavage furrow at the periphery of the cell up to the completion of the cytokinesis takes about three minutes and a half. The time taken for the cleavage to reach the spindle is about one minute and the time taken for the cleavage to cut through the spindle is about two minutes and a half. Such an extreme rapidity of the process as observed here does not appear to have been recorded previously among Diatoms. The only previous record appears to be by Gross (1937-38, p. 5) on *Ditylum Brightwellii*, where the process according to him takes 30 minutes.

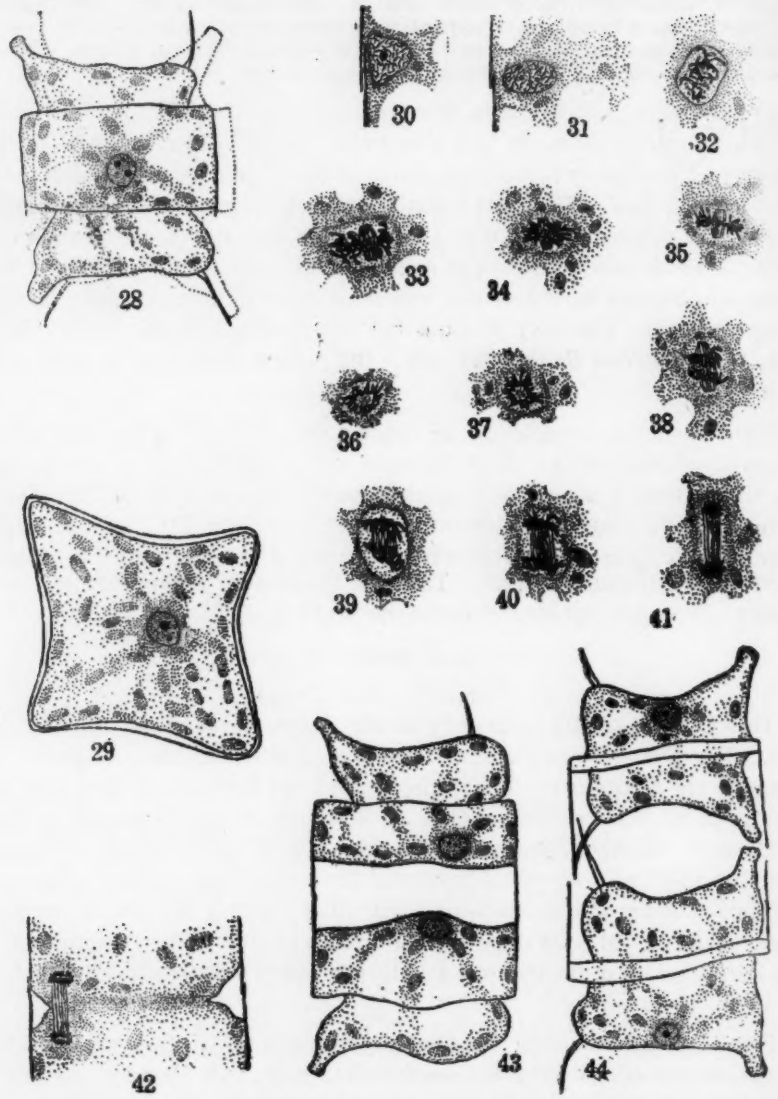
Soon after the completion of cytokinesis, each daughter protoplast secretes a silicified valve. After the new vavles are secreted by the protoplast the transverse septa start as small protuberances from the periphery of the new valves and grow inwards (Text-Figs. 26 and 27). In the early stages of their formation the growing septa are connected with the nucleus by definite cytoplasmic strands. The time taken after completion of cytokinesis to form new valves and transverse septa is about 3 to 4 hours.

2. *Triceratium dubium* Brightwell
(= *Biddulphia dubia* Cleve)

This Diatom occurs commonly in the marine plankton of the Madras coast. The Diatom was isolated from the plankton catch and grown in cultures in the laboratory. The methods employed for isolating and growing the Diatom in cultures were the same as those used for *Cyclotella Meneghiniana* (Iyengar and Subrahmanyan, 1944, pp. 127–28), only, instead of pond water, candle-filtered sea-water was used for making the culture solutions. Both agar and liquid cultures were tried. The Diatom did not thrive well in agar cultures, but grew very well in liquid cultures. The Diatom multiplied fairly rapidly in the cultures and formed very long chains (Pl. XXIX, Fig. 1).

For fixing and staining the Diatom the methods* employed were the same as those used for *Terpsinoë musica* Ehrenberg, only sea-water was used for making up the fixing solution. Of the several fluids employed only Flemming's weak solution and PFA_3 were found most suitable. The Diatom

* The methods are given in detail under *Terpsinoë musica*, only the deviations are indicated here and in the following forms.



Text-Figs. 28–44. *Triceratium dubium*.—Fig. 28. Cell in girdle view; (the dotted line shows cell outline in the lower focus). Nucleus in resting condition. Fig. 29. Cell in valve view. Figs. 30–31. Early prophase. Fig. 32. Late prophase. Figs. 33–34. Early metaphase with the spindle and the chromosomes arranged round it at the equatorial region. Fig. 35. Metaphase. Figs. 36–37. Metaphase in valve view. Note ring-like arrangement of the chromosomes.

Fig. 38. Late anaphase. Fig. 39. Early anaphase; note nuclear membrane still persisting. Figs. 40–42. Late anaphase; note beginning of cytokinesis in Fig. 42. Fig. 43. The two daughter protoplasts just separated after cytokinesis. Fig. 44. The two daughter cells after formation of new valves, horns and processes. All figs. ×1200.

chains were pipetted out from the cultures and transferred to the fixing fluid. In all the cases the chains broke up into the individual cells in the fixing fluid. Staining in iron-alum hæmatoxylin gave the best results. Bleaching for 15 minutes, mordanting for one hour and staining for three hours were found quite sufficient for this Diatom.

Vegetative cell.—The cells of *Triceratium dubium* are box-shaped. In girdle view, two angles show a stout horn-like prolongation and the other two angles show each a short, blunt process (Text-Fig. 28). In valve view the Diatom is usually four-sided (Text-Fig. 29) but three-sided and five-sided valves also were observed (*cf.* Hustedt, 1930, p. 807, Fig. 469). The valves and the girdles are strongly sculptured. The cytoplasm forms a lining layer in which are imbedded numerous yellowish brown chromatophores. The nucleus usually lies in the centre of the cell suspended by cytoplasmic strands (Text-Figs. 28 and 29).

Mitosis.—Material fixed during the early hours of the morning (4–6) A.M. showed plenty of division figures. The resting nucleus is spherical and shows one, rarely two or three nucleoli and a lightly stained reticulum. (Text-Figs. 28 and 29).

Just prior to division the nucleus, which usually lies at the centre of the cell in the resting condition, moves towards one side of the cell and remains in the lining layer of cytoplasm where all the stages of division take place. A similar behaviour of the nucleus was observed by Cholnoky (1933) in *Melosira arenaria* and by the writer in *Navicula halophila* (unpublished). The cell about this time increases somewhat in volume, the valves loosening a little to allow for this increase in size.

During prophase the reticulum is stained deeper and thin chromosomal threads appear in the nuclear cavity (Text-Figs. 30 and 31). In late prophase the chromosomes become slightly thicker and shorter and are distributed more or less evenly in the nuclear cavity (Text-Fig. 32).

During metaphase, the spindle, which is barrel-shaped, becomes evident (Text-Figs. 33 and 34); and the chromosomes, which are long and variously shaped, are seen arranged in a compact ring round the spindle at its equatorial region (Text-Figs. 35–37). The distribution of the chromosomes is more or less even in the ring unlike in *Terpsinoë musica* where they are slightly unequally distributed. The nucleolus disappears at about

early metaphase. The nuclear membrane also disappears about this time. But occasionally it was seen persisting till anaphase (Text-Fig. 39).

The exact chromosome number could not be accurately determined since the chromosomes are many and very compactly arranged. As far as the writer was able to count, the number of chromosomes appeared to be 26–30 ($2n$).

In anaphase the daughter chromosomes separate and move towards the poles of the spindle (Text-Figs. 38, 39 and 40). The chromosomes in anaphase lie somewhat parallel to the axis of the spindle. After reaching the poles of the spindle the chromosomes contract; at this stage it is very difficult to make out the outline of the individual chromosomes (Text-Figs. 41 and 42). During telophase the daughter nuclei are organised. The daughter nuclei which are at first close to each other (Text-Fig. 43) finally move to the centre of the respective cells. Occasionally they move to the opposite ends of the cells and remain in the parietal layer of the cytoplasm (Text-Fig, 44).

Cytokinesis.—During anaphase cytokinesis commences. In optical section it is seen to commence as a small cleavage furrow near the periphery of the cell and the furrow progresses centripetally (Text-Fig. 42) and finally the protoplast is divided into two. As soon as the fission is complete, the daughter protoplasts appear to contract a little leaving a fairly wide space in between (Text-Fig. 43). A similar phenomenon was observed by Gross (1937/38) in *Ditylum Brightwellii.* After this the new valves with their characteristic markings and processes are soon formed.

The daughter cells generally do not separate immediately after division but remain attached to each other by means of a mucilage pad formed at one of the angles. In this way long zig-zag chains are formed, the mucilage pads generally being formed in diagonally opposite angles in alternate cells.

3. *Biddulphia mobiliensis* Bail.

The only previous account of this Diatom appears to be by Peragallo in 1907. Unfortunately the writer could not get Peragallo's original paper. But a brief summary of Peragallo's observations together with his figures of cell division is given by Oltmanns (1922, Bd. I, p. 128). Peragallo's observations regarding nuclear division as far as could be judged from the account given by Oltmanns appear to be meagre and not quite satisfactory. Oltmanns states finally that further observations should be awaited on this Diatom.

This Diatom was found in the marine plankton of the Madras coast. It was isolated from the other forms and grown in cultures in the laboratory.

The same procedure was followed as for the previous Diatom (*Triceratium dubium*) for isolating the Diatom and growing it in cultures in the laboratory. For fixing and satining, the same methods as those used for the previous forms were employed. Fixing in PFA_3 and staining in iron-alum hæmatoxylin gave the best results.

Vegetative cell.—The cells of *Biddulphia mobiliensis* Bail. are box-shaped in girdle view and elliptical in valve view. In girdle view the corners are prolonged into horns and between the horns two long spines are situated on a slightly raised portion. The cells at times form long or short chains. The cytoplasm forms a lining layer and the nucleus is suspended by cytoplasmic strands at the centre of the cell. Numerous disc-shaped and somewhat slightly lobed brownish-yellow chromatophores are seen distributed at the periphery of the cell (Text-Fig. 45).

Mitosis.—Material fixed between 4 and 6 A.M. showed plenty of division figures, but dividing cells were also seen frequently at other times of the day in the cultures. The resting nucleus is spherical and shows one, rarely two nucleoli and a well-stained reticulum. Prior to division the cell increases in volume, the valves loosening slightly to allow for this increase. The nucleus during division is seen situated in the parietal layer of cytoplasm as in the previous form.

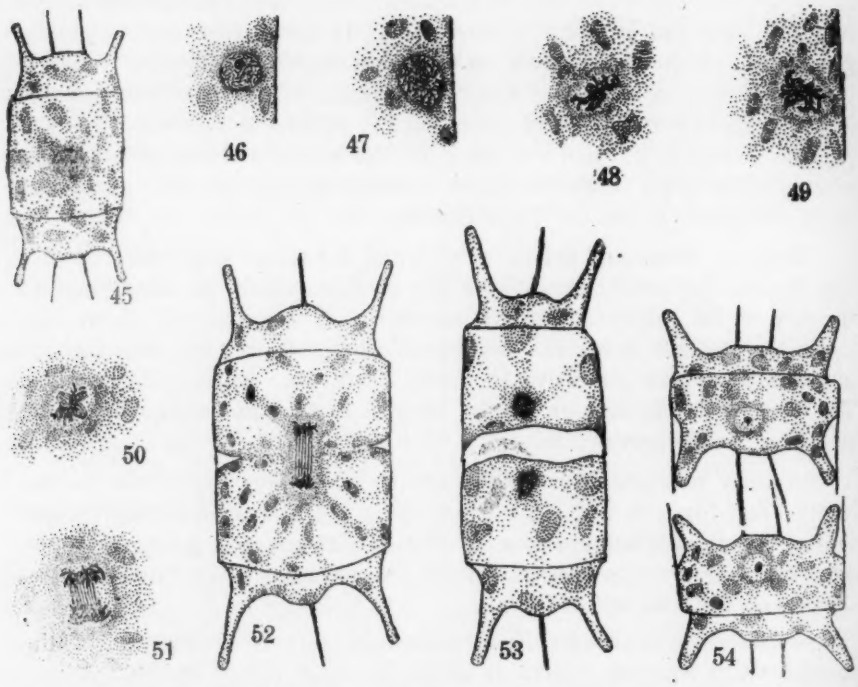
In early prophase thin chromosomal threads are seen in the nuclear cavity (Text-Figs. 46 and 47). In late prophase the chromosomes become shorter and thicker and are seen distributed in the nuclear cavity (Text-Fig. 49). The nucleolus and nuclear membrane disappear about late prophase, sometimes early metaphase.

During early metaphase the chromosomes move to the equator of the spindle which becomes evident at about this stage (Text-Fig. 48). A little later the chromosomes are seen arranged in a ring at the equator of the spindle (Text-Fig. 50). Good polar views of metaphase were not observed but the ring-like arrangement could be clearly made out by proper focussing.

The number of chromosomes could not be determined with certainty owing to the compact arrangement of the chromosomes. Their number appeared to be about 20 ($2n$).

In anaphase the daughter chromosomes move to the poles of the spindle (Text-Figs. 51 and 52) and in telophase the daughter nuclei are organised.

Cytokinesis.—Cytokinesis begins during anaphase as in the previous cases. It is seen in optical section as a clevage furrow, which advances in a centripetal manner and cuts the protoplast into two (Text-Figs. 52 and 53). Cytokinesis took place even more rapidly than in *Terpsinoë musica*. The time taken from the commencement of the furrow at the sides of the cell to the completion of the division of the protoplast was less than 30 seconds. The division was so rapid that it was impossible to make any camera lucida drawings of the stages.



Text-Figs. 45–54. *Biddulphia mobiliensis.*—Fig. 45. Vegetative cell in girdle view. Figs. 46–47. Early prophase. Fig. 48. Early metaphase. Note the spindle. Fig. 49. Late prophase. Fig. 50. Late metaphase. Fig. 51. Anaphase. Fig. 52. Anaphase; note beginning of cytokinesis. Fig. 53. Cytokinesis completed. Fig. 54. Daughter cells after formation of valves and spines and horns. Figs. 45 and 54, ×760; rest, ×1170.

As soon as the cleavage is completed the daughter protoplasts are separated as in the previous form (*Triceratium dubium*) by a wide empty space between them. The exact significance of this space is not clear. Gross (1937/38, p. 5) states in the case of *Ditylum Brightwellii* that " the fact that the two daughter cells together fill a space far smaller than the original

parental one suggests the existence of a strong bipolar tension prior to and during the division."

The development of the characteristic horns and spines of this Diatom (Text-Fig. 54) has been described in detail by Iyengar and Subrahmanyan (1944 *a*).

4. *Achnanthes inflata* (Kutz.) Grun.

This Diatom occurs as an epiphyte on *Cladophora* and *Pithophora* along with *Terpsionoë musica* in a pond in the Agri-Horticultural Gardens at Madras. The Diatom often forms short chains. The basal cell is attached to the algal filament by a mucilage pad at one corner (Text-Fig. 55).

The Diatom was fixed both in the field and in the laboratory. Division figures were found in plenty in material fixed between 11 P.M. and 2 A.M. The material was fixed in the several fluids used for *Terpsinoë*, but only Schaudinn's sublimate-acetic-alcohol and PFA_3 gave good results. The material was washed and stained in iron-alum hæmatoxylin in the same manner as the previous forms.

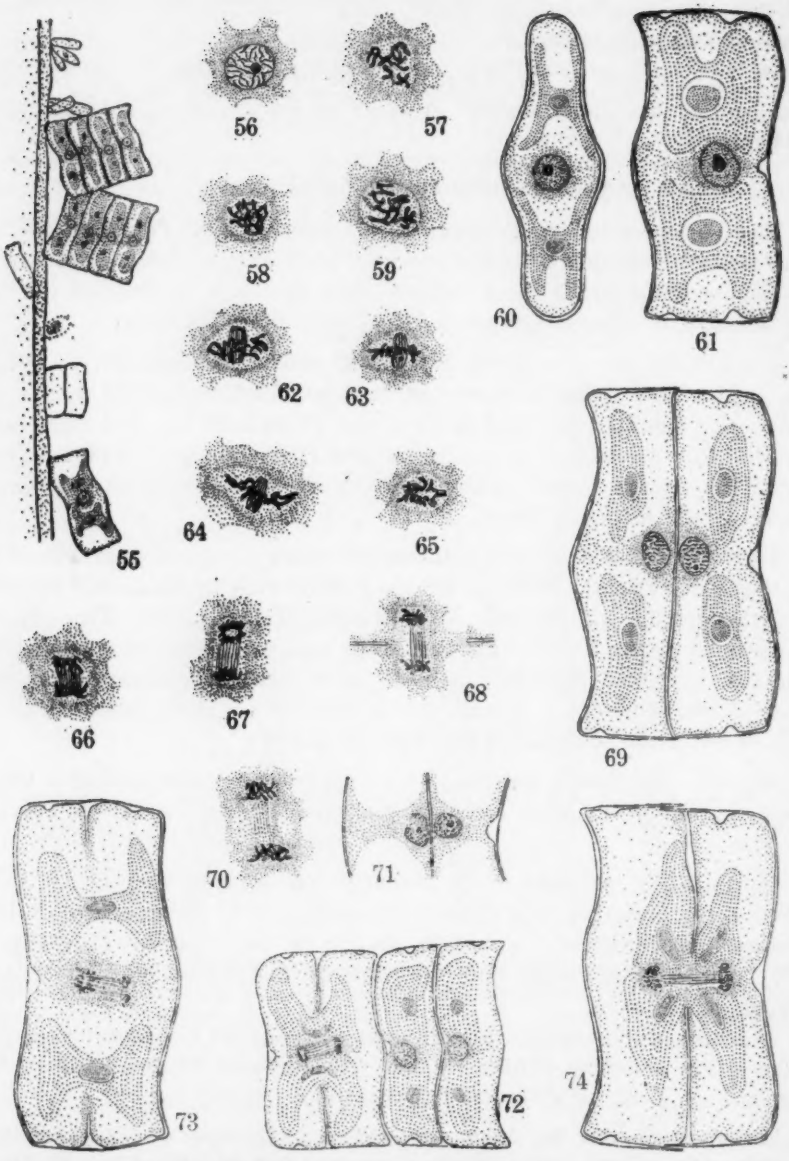
Vegetative cell.—The cells of *Achnanthes inflata* are rectangular with the two long sides curved (Text-Fig. 61) and in valve view are linear and bulged out in the middle with broadly rounded poles (Text-Fig. 60). Two plate-like chromatophores lie on either side of the nucleus. Each chromatophore possesses a pyrenoid (Text-Fig. 60). In valve view the chromatophore is H-shaped (Text-Fig. 60). The nucleus is situated in a dense mass of cytoplasm at the centre of the cell (Text-Figs. 60 and 61).

Mitosis.—The resting nucleus shows a prominent nucleolus and a well stained reticulum. In some cases the nucleolus appears vacuolated (Text-Fig. 60).

During early prophase thin chromosomal threads are seen in the nuclear cavity (Text-Fig. 56). These later contract and in late prophase 12–16 somewhat long chromosomes are seen lying in the nuclear cavity (Text-Figs. 57–59). By about this stage both the nucleolus and the nuclear membrane disappear completely.

While the prophasic changes are taking place the cell increases in volume by the valves loosening a little so that when division is completed each daughter cell is only slightly less broad than the mother cell.

In early metaphase the chromosomes move to the equator of the spindle (Text-Fig. 62) and form a compact ring at metaphase (Text-Figs. 63–65). The spindle is barrel-shaped as in the previous cases and is first noticeable in early metaphase (Text-Fig. 62).



Text-Figs. 55–73. *Achnanthes inflata*.—Fig. 55. Habit. Note the cells forming chains and the attachment mucilage pad. Fig. 60. Valve view of cell. Note the H-shaped chromatophores with pyrenoid. Nucleus in resting condition. Fig. 61. Girdle view of cell; nucleus

in resting condition. Fig. 56. Early prophase. Figs. 57–59. Late prophase. Note disappearance of both nucleolus and nuclear membrane. Figs. 62–64. Metaphase. Fig. 65. Metaphase, polar view (slightly oblique). Figs. 66–68, 70. Anaphase. Cytokinesis well advanced in Fig. 68. Fig. 73. Anaphase. Note beginning of cytokinesis. Fig. 71. Daughter nuclei organised. Fig. 69. Cell after completion of cytokinesis. Note one nucleus and two chromatophores in each cell. Fig. 72. One cell (left) showing cytokinesis and division of chromatophores and elongated pyrenoids; cell on the right completely divided. Fig. 74. Cell undergoing division; chromatophores and pyrenoid just divided; cytokinesis not complete; nuclei in telophase. Fig. 55, ×270; Fig. 72, ×750; rest, ×1150.

In anaphase the daughter chromosomes move to the poles of the spindle (Text-Figs. 66, 67, 68 and 70) and then contract. During telophase the daughter nuclei are organised (Text-Figs. 74.)

Cytokinesis.—Cytokinesis begins during anaphase as a small cleavage furrow which progresses in a centripetal manner and finally cuts the cell into two (Text-Figs. 72, 73, 74 and 69). During division, the chromatophores and the pyrenoids elongate and divide by constriction (Text-Figs. 72–74). After the division of the cytoplasm and the chromatophores is completed, new valves are formed (Text-Fig. 72, right cell).

The observations agree in a general way with those of Gemeinhardt (1925) on *Achnanthidium brevipes* (Ag.) Cl. But Gemeinhardt did not observe the several stages of prophase and the spindle clearly. Again the chromosomes in his form are short and dot-like whereas in the present form they are long and thread-like.

5. *Pleurosigma angulatum* (Quekett) W. Smith

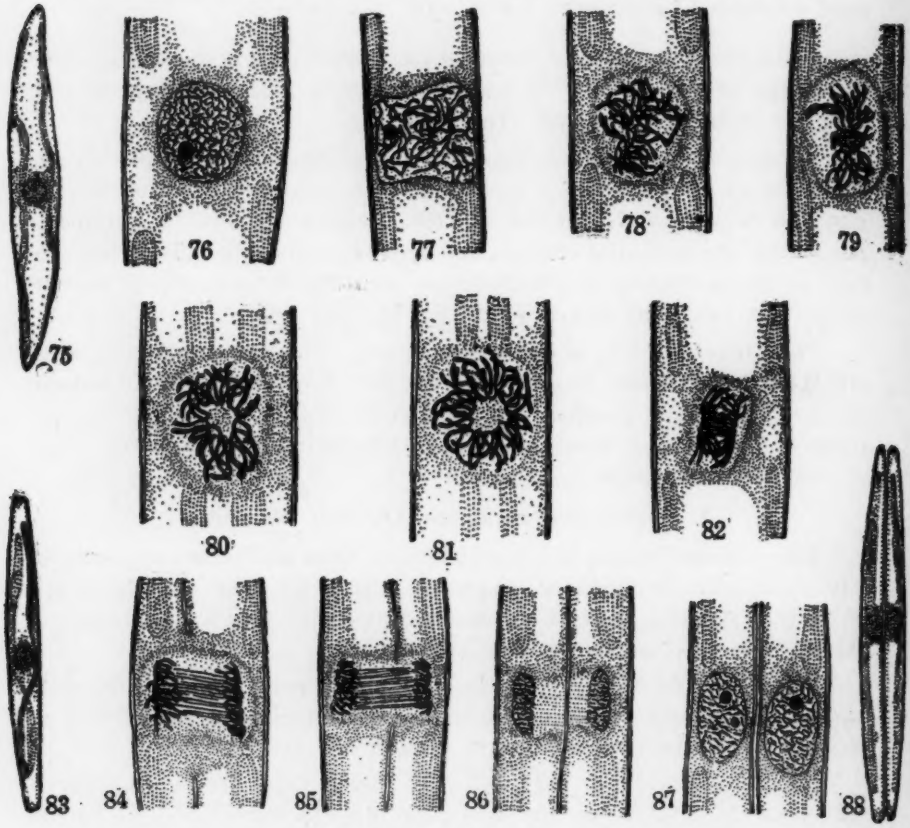
This pennate Diatom is a brackish-water form and occurs in plenty as a brownish scum on the moist sand at the edge of the water near the mouth of the river Adyar at Madras. It occurred more or less in a pure formation except for a few smaller pennate Diatoms.

Fixing in PFA_3 made up with the river water and staining in iron-alum hæmatoxylin proved quite satisfactory. Nuclear figures were found in plenty in the material fixed between 5 and 7 P.M.

Vegetative cell.—The cells of *Pleurosigma angulatum* appear like a drawn out 'S' in valve view and rectangular in girdle view (Text-Figs. 75 and 83). The cells are several times longer than broad. The chromatophores are ribbon-shaped and lie more towards the girdle side in the resting cell. The large nucleus is situated at the centre of the cell in a dense mass of cytoplasm (Text-Figs. 75 and 83).

Mitosis.—The resting nucleus shows a single nucleolus and a deeply stained reticulum (Text-Fig. 76). The reticulum in this Diatom is more strongly stained than in all the previous forms studied by the writer.

During prophase a number of long thread-like chromosomes is seen lying in the nuclear cavity (Text-Fig. 77). The spindle is barrel-shaped and becomes evident during early metaphase (Text-Figs. 78 and 79). The chromosomes move towards the equator of the spindle and are seen divided longitudinally (Text-Fig. 79). They are then arranged uniformly in a ring around the spindle at metaphase. Only polar views of the spindle were



Text-Figs. 74–88. *Pleurosigma angulatum*.—Fig. 75. Cell in valve view. Fig. 83. Cell in girdle view. Fig. 76. Resting nucleus. Fig. 77. Prophase. Note long thread-like chromosomes. Fig. 78. Early metaphase; nuclear membrane and nucleolus disappeared. Fig. 79. Metaphase. Note the spindle; the chromosomes already divided. Figs. 80–81. Metaphase, in polar view. Note arrangement of chromosomes in a ring and the spindle fibres seen as dots inside the chromosome ring. Fig. 82. Early anaphase. Fig. 84. Anaphase. Cytokinesis well advanced. Fig. 85. Late anaphase. Cytokinesis advanced still further and very near the spindle. Fig. 86. Telophase. Cytokinesis completed. Fig. 87. Daughter nuclei organised and new valves formed. Fig. 88. Completed daughter cells. Figs. 75, 83, 88, ×420; rest, ×1200.

seen. Side view of metaphase was not observed. Inside the chromosome ring could be seen a number of dots representing the spindle fibres in polar view (Text-Figs. 80 and 81; Pl. XXIX, Fig. 5).

An exact chromosome count could not be made either at metaphase or the earlier stages as the chromosomes were long and many and very compactly arranged. The number of chromosomes appears to be about 40 (2 *n*).

In anaphase the daughter chromosomes move to the poles of the spindle, the spindle lengthning as the daughter chromosomes move farther apart (Text-Figs. 82 and 84). The chromosomes then contract and the outline of the individual chromosomes becomes very difficult to make out (Text-Fig. 85). In telophase the daughter nuclei are organised (Text-Fig. 86).

Cytokinesis.—Cytokinesis commences during anaphase as a tiny cleavage furrow advancing to the centre and finally cutting the cytoplasm into two in the valvar plane (Text-Figs. 84–86). Later two new valves are secreted (Text-Figs. 87 and 88).

Discussion

Nucleolus.—Schmidt (1927) while investigating nuclear division in ***Biddulphia sinensis*** came to the conclusion that in the resting nucleus all the chromatic substance was situated in the nucleolus and that the outer nucleus (reticulum area) contained no chromatin material. The chromosomes according to him were derived solely from the nucleolar substance.

Cholnoky (1927 *b*) held in the case of *Diatoma vulgare* that a wandering of chromatin substance takes place from the nucleolus to the chromosomes.

Geitler (1928, p. 500) after severely criticising Schmidt's (*l.c.*) view, holds that the nucleolus does not represent so to say a superfluous reserve substance for the formation of the chromosomes, but that the nucleoli of Diatoms are quite comparable to those of the Metaphyta.

Later on, Cholnoky (1933) after investigating the nuclear division in *Melosira arenaria*, states that the chromosomes are well developed long before the nucleolus disappears and that the nucleolus is not chromatic in nature. He also states that his former view (*l.c.* 1927 *b*) regarding the wandering of chromatin from the nucleolus to the chromosomes was based on the then generally accepted ideas.

Schmidt reinvestigated ***Biddulphia sinensis*** in 1933 and as a result of his observations withdrew his former opinion that the chromosomes were derived from the nucleolus.

The writer's observations on the nuclear division of the five Diatoms investigated clearly show that the chromosomes never take their origin from the nucleolus. They always take their origin from the reticulum. In all the five Diatoms he found that the chromosomes were already well formed in the nuclear cavity (outer nucleus) while the nucleolus is still in tact. Again, Feulgen's reaction was tried by the writer for all the five forms several times. The nucleolus in all the five forms always gave a negative reaction, while the reticulum (in the resting nucleus) and the chromosomes (in the division figures) gave a positive reaction. The result of the Feulgen's reaction clearly showed that the nucleolus is not chromatic in nature.

Spindle.—There is a certain amount of controversy regarding the origin of the spindle. According to Lauterborn (1896), the spindle in *Surirella Capronii* is formed outside the nucleus by a round body derived from the centrosome. This body becomes a cylindrical spindle and penetrates into the nuclear cavity during the prophase of mitosis. But Belar (1926, p. 285) considers such an origin of the spindle as not probable when compared with what takes place in other forms with a central-spindle mitosis.

Ikari (1923) also thought that the spindle is extra-nuclear in origin. He observed in *Coscinodiscus subbuliensis* the spindle lying in close contact with the nucleus but outside it. Cholnoky (1933 *b*, p. 704) states that Ikari probably saw the 'normal' centrosomes with radiation, but interpreted them according to Lauterborn.

Karsten (1900, 1912) found during reduction division in *Surirella splendida* (*S. Saxonica*) that the single centrosome present elongates and forms the spindle.

Geitler (1927) found in *Cocconeis placentula* var. *klinoraphis* the centrosome penetrating into the nuclear cavity during prophase and forming the spindle.

It may be mentioned in this connection that centrosomes have been recorded by Cholnoky (1933 *a*) in *Cymbella cistula, Gomphonema capitatum* and *Gyrosigma accuminatum.* In the first form the centrosomes were observed at the poles of the spindle during reduction division.

Cholnoky (1933 *b*) states in the case of *Melosira arenaria* that he was not able to establish the exact origin of the spindle. He could see the spindle only in late prophase stages.

In the five forms dealt with in the present paper the writer was not able to find a definite centrosome. The spindle in every case was first seen in early metaphase and became more definite in metaphase. Its exact origin,

however, could not be traced. Again, no evidence for an extra-nuclear origin of the spindle was found though numerous preparations were very carefully examined.

It may be mentioned in this connection, however, that in *Navicula halophila* (Subrahmanyan, unpublished), a darkly stained body was seen close to the nucleus during prophase. This body could not be seen during early metaphase and later stages. Nor could it be seen in the resting nucleus though numerous preparations were carefully searched. The only stages during which it could be seen are mid- and late prophase. This dark body showed a close resemblance to the centrosome in *Cocconeis placentula* var. *klinoraphis* as figured by Geitler (1927, Taf. 13, Figs. 4 and 5). According to Geitler, the centrosome in this Diatom penetrates into the nuclear cavity and then forms the spindle. In *Navicula halophila* the writer could not observe the penetration of the dark body into the nuclear cavity. The fact that the disappearance of the dark body is soon followed by the appearance of the spindle inside the nuclear cavity in early metaphase would appear to suggest that here also the spindle is formed by the centrosome as in *Cocconeis placentula* var. *klinoraphis* as recorded by Geitler (1927). But until the actual entry of this dark body into the nuclear cavity and its modification into the spindle inside the nucleus are observed, nothing further could be definitely stated regarding this matter.

Cytokinesis.—Almost all the previous observations on the process of cytokinesis in Diatoms are based on fixed material. Gross (1937-38) appears to be the only worker so far to have followed cytokinesis in a living Diatom (*Ditylum Brightwellii*). He found that the process took 30 minutes to complete. In the present investigation the writer followed the cytokinesis in the living specimens of two Diatoms, *viz.*, *Terpsinoë musica* and *Biddulphia mobiliensis*. In both these Diatoms the process was surprisingly rapid. In *Terpsinoë musica* the process took only three and a half minutes to complete, while in *Biddulphia mobiliensis* the process took hardly 30 seconds to complete. It may be mentioned, however, that the cytokinesis in *Terpsinoë* was not uniformly rapid throughout. It was very rapid until the spindle region was reached. But after reaching the spindle, the cytokinesis was slowed up very much as if some resistance was being offered by the spindle. It took just a minute to reach the spindle region but took nearly two and a half minutes to cut through the spindle. In the case of *Biddulphia*, however, this slowing up of the process when the spindle region was reached was not noticeable. The process appeared to be fairly uniformly rapid throughout. It would be interesting if more Diatoms are studied in their living condition as regards their cytokinesis.

Summary

Nuclear and cell division was followed in detail in five Diatoms, three belonging to the Centrales (*Terpsinoë musica*, *Triceratium dubium* and *Biddulphia mobiliensis*) and two to the Pennales (*Pleurosigma angulatum* and *Achnanthes inflata*).

In the resting nucleus are seen a well-developed reticulum and one or occasionally two or three nucleoli. The chromosomes become well differentiated during prophase and are distributed evenly inside the nucleus.

The nucleolus in all the five forms invariably disappears in late prophase and reappears when the daughter nuclei are organised.

The nuclear membrane generally disappears about metaphase in *Terpsinoë musica*, and about late prophase or about early metaphase in the other forms. The nuclear membrane was observed to persist till anaphase only in one case in *Triceratium dubium*.

The spindle makes its appearance during early metaphase. The chromosomes at metaphase are arranged in a ring round the spindle.

After anaphase and telophase the daughter nuclei are organised.

The chromosomes do not originate from the nucleolus, but are derived from the reticulum. The nucleolus gives a negative reaction with Feulgen stain, while the reticulum and the chromosomes give a positive reaction.

The number of chromosomes appeared to be about 28 ($2n$) in *Terpsinoë musica*, about 26–30 ($2n$) in *Triceratium dubium*, about 20 ($2n$) in *Biddulphia mobiliensis*, about 12–16 ($2n$) in *Achnanthes inflata* and about 40 ($2n$) in *Pleurosigma angulatum*.

Cytokinesis was followed in living specimens of *Terpsinoë musica* and *Biddulphia mobiliensis*. The process in these two Diatoms is extremely rapid, the time taken for the whole process being three and a half minutes in *Terpsinoë* and about thirty seconds in *Biddulphia*.

In conclusion, the writer wishes to express his great indebtedness to Prof. M. O. P. Iyengar, M.A., Ph.D. (Lond.), F.L.S., for his constant guidance and valuable help throughout the course of the present investigation. The writer's sincere thanks are also due to the authorities of the University of Madras for the award of a studentship during the tenure of which this investigation was carried out.

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Explanation of Plate XXIX

Fig. 1. *Triceratium dubium.*—Portion of a chain of the Diatom (living material). ×500.

Fig. 2. *Terpsinoë musica.*—Shows habit. Diatom forming zig-zag chains attached to *Cladophora.* ×34.

Fig. 3. *Terpsinoë musica.*—One girdle side of a living cell just before beginning of cytokinesis. Arrow points to the region where furrowing will take place. ×1200.

Fig. 4. *Terpsinoë musica.*—A portion of the Diatom chain ×62.

Fig. 5. *Pleurosigma angulatum.*—Metaphase, polar view. Note chromosomes arranged round the spindle. ×1500.

Fig. 6. *Terpsinoë musica.*—The same specimen shown in Fig. 3 killed in osmic acid when cytokinesis just started. Arrow points to the furrow. ×1200.

Fig. 7. *Terpsinoë musica.*—Metaphase. Note barrel-shaped spindle. ×1500.

Fig. 8. *Terpsinoë musica.*—Anaphase. ×1500.

Fig. 9. *Terpsinoë musica.*—Late prophase seen from valve view. Note long thread-like chromosomes. ×1500.

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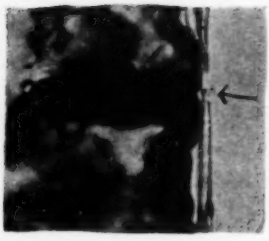

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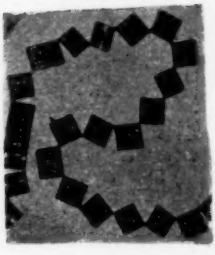

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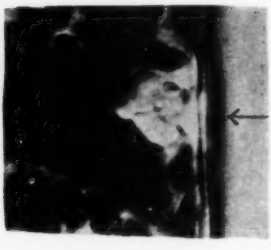

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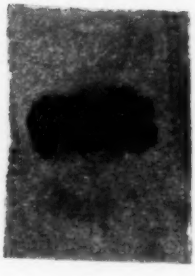

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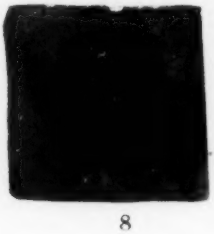

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THE CO-RELATION OF THE WEST COAST (MALABAR & SOUTH KANARA) FISHERIES WITH PLANKTON AND CERTAIN OCEANOGRAPHICAL FACTORS*

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Received September 6, 1945
(Communicated by Prof. S. G. Manavala Ramanujam, F.A.SC.)

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1. Introduction

It is well known that fishes depend either directly or indirectly upon the nature and availabilty of planktonic organisms in the seas. Animal life in the sea subsists on macro and micro plankton, consisting of small animalcules and minute plant organisms; the latter depend for their food on various chemical substances such as phosphates, nitrates, etc. One can, therefore, partly attribute the fluctuations of the Fisheries to the hydrographical variations such as the fluctuations of the physical and chemical factors and the consequential changes in the productivity of plankton. In addition to hydrography, ecology or the study of living things in relation to their environment is equally important in fisheries research. In this paper, an attempt is made to co-relate the hydrographical factors, ecological findings and fishery landings at Calicut Fishing Centre with one another. The scope of the study is therefore necessarily narrow but nevertheless it, perhaps, furnishes key to similar problems in other fishing centres all over the West Coast. The present investigation covers a period of 5 years from 1938 to 1942.

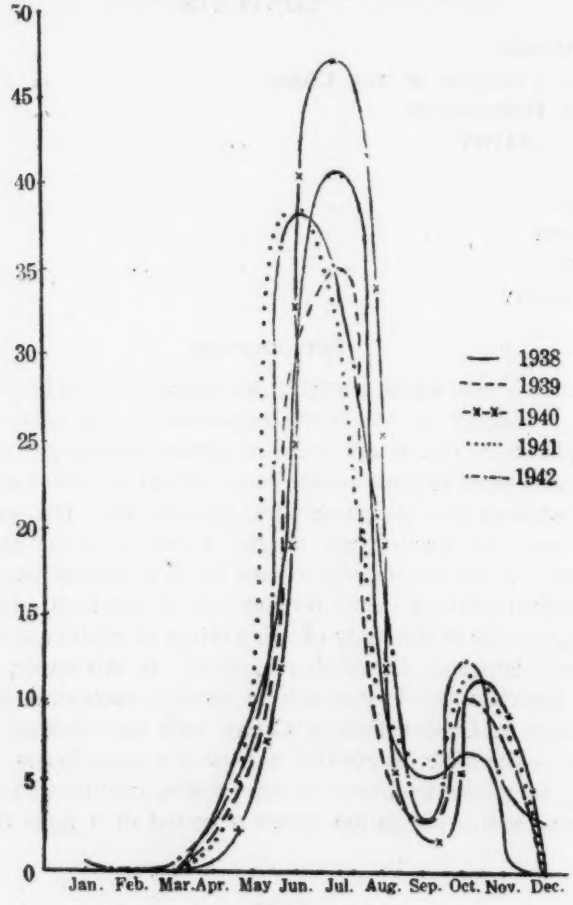
* With the kind permission of the Director of Industries and Commerce, Madras.

2. Physical Features of the Coast

There are two rivers, one the Korapuzha emptying into the sea north of Calicut and the other, the Beypore, south of Calicut (Lat. 11–18 N. and Long. 73–43 E.). Calicut is taken as an indicator for the whole of the West Coast of Madras Presidency and the important physical features of this area are:—

(1) The narrow belt of low land between the sea and the Western Ghats and

(2) The shortness of the rivers that run into the sea.



Rainfall

During the rainy season of the S.W. Monsoon, these rivers discharge into the sea large quantities of silt and organic matter, both in solution and in suspension. The seasonal climatic changes consist of a wet season from June to September, a short hot and dry transition period in September and October, the cool dry N.E. Monsoon period from November to February and the hot dry season characterised by calms and light breeze from March to May.

The rainfall is very heavy during June and July especially on the western slopes of the Ghats from where large volumes of rainwater rush down through the short rivers, carrying plenty of nutrient salts and organic debris into the sea. The total amounts of rainfall during the years 1938, 1939, 1940, 1941 and 1942 were 117·7455, 98·382, 116·9, 118·09, and 111·8 inches respectively, the maximum being in the months of May, June and July. Again in the months of October and November there is a second peak of rainfall which though not so heavy as the other is still bad enough to create unsettled weather conditions to restrict fishing (*vide* graph).

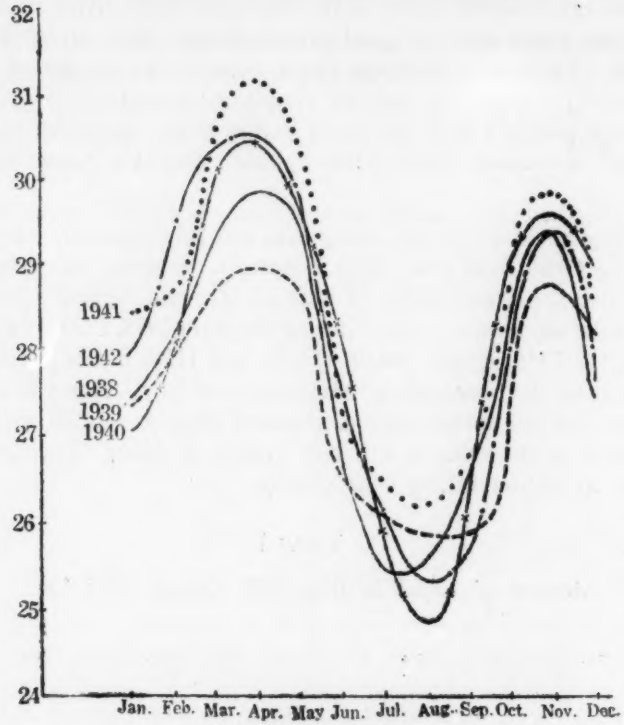
TABLE I

Amount of rainfall at West-Hill, Calicut, 1938–1942

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1938 ..	..	..	·08	9·9815	5·13	30·829	41·245	16·267	6·54	7·233	·44	..
1939 ..	..	..	..	3·21	·112	28·14	35·68	9·82	3·35	10·624	7·23	..
1940 ..	..	..	..	·15	8·83	22·47	47·6	20·12	1·73	11·12	4·88	..
1941 ..	·29	..	..	2·09	20·34	38·14	14·621	16·21	6·65	11·32	8·43	..
1942 ..	..	..	·14	3·73	6·27	37·44	34·11	10·71	2·91	12·34	2·87	3·28

3. SURFACE TEMPERATURE

The surface temperature of sea-water has been found to reach the maximum of about 31° C. in March and April though occasionally in the month of May also, if the outbreak of monsoon is a little delayed. In June, July, August and September, it is low falling down to 24·8° C. There is another sharp rise in October from whence the temperature is gradually in the increase (*vide* Table II). The lowering of the temperature during the months of June, July and August is due to the rains that break with the S.W. Monsoon and to the consequent reduction in the number of hours of sunshine.



Surface Temperature

TABLE II

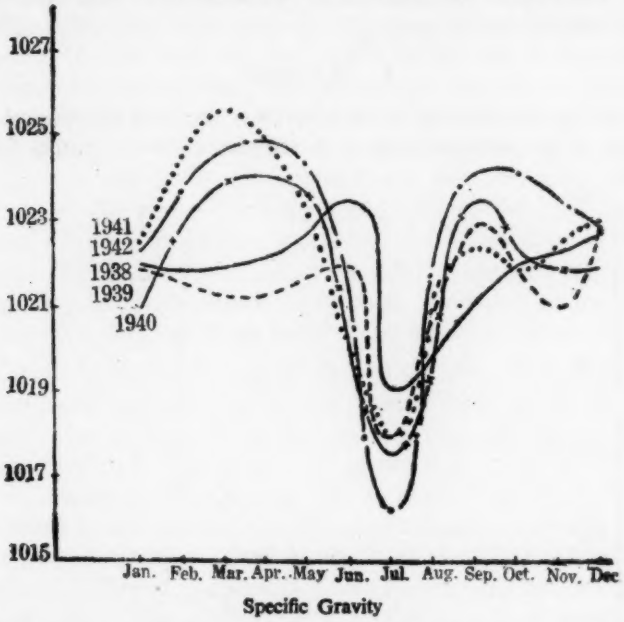
Surface Temperature of sea-water at West-Hill 1938–42

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1938 ..	27·45	27·95	29·75	29·75	29·8	27·35	25·4	25·55	26·7	27·6	29·5	27·5
1939 ..	27·35	27·9	28·7	29·95	29·	26·7	26·25	26·55	25·85	28·5	29·5	27·7
1940 ..	27·05	28·10	28·45	30·5	30·1	28·15	25·75	24·85	26·3	29·25	29·65	28·9
1941 ..	28·45	28·65	30·25	31·20	29·55	27·30	26·55	26·2	26·75	28·25	29·90	28·80
1942 ..	27·90	29·10	30·25	30·5	29·55	27·45	26·2	25·35	25·65	28·10	28·80	28·20

4. SPECIFIC GRAVITY

The specific gravity of the inshore water is the lowest in the month of July during the quinquennium under consideration. The mean specific gravity of the sea-water in different months of the five years is given in the table annexed. Taking only the month of July when the lowest specific

gravity occurs along with the heaviest rainfall in 1938 it was 1019·0 falling downwards to 1018·0 in 1939 and again to 1017·5 in 1940. In 1941 it



rose to 1018·0 and in 1942 it went down to 1016·1. From August onwards there is a gradual increase in specific gravity till May.

There is a slight fall in the specific gravity in the month of November or December on account of the rain-fall caused by the N. E. Monsoon.

TABLE III

Specific Gravity of the sea-water at West-Hill 1938–42

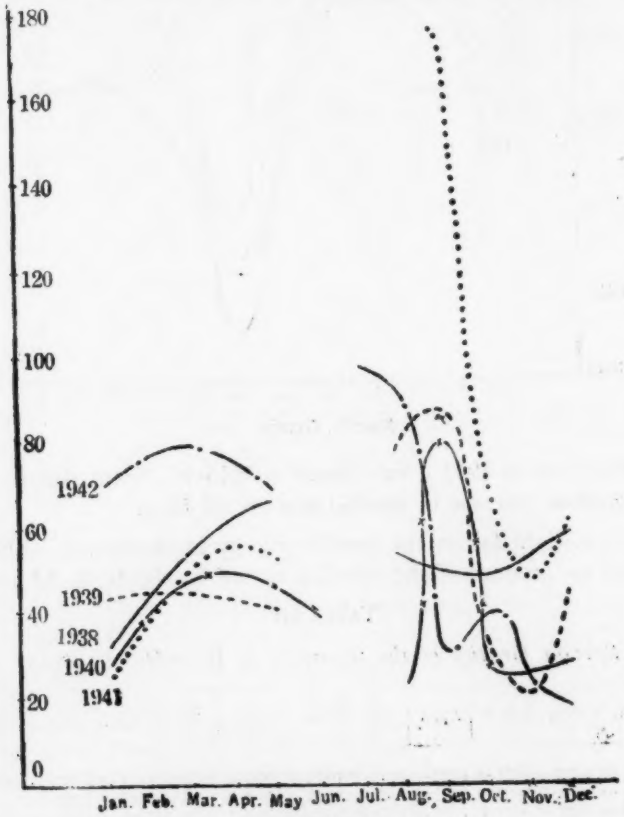
Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1938 ..	1022·0	1022·0	1021·8	1022·0	1022·0	1022·0	1019·0	1020·0	1021·0	1022·0	1022·3	1022·5
1939 ..	1023·0	1023·0	1023·0	1021·0	1023·0	1023·0	1018·0	1021·0	1023·0	1022·0	1021·0	1023·0
1940 ..	1020·9	1022·0	1023·8	1023·0	1023·7	1023·3	1017·5	1020·0	1023·5	1022·5	1022·0	1022·0
1941 ..	1022·5	1023·0	1025·6	1023·4	1023·5	1022·0	1018·0	1021·0	1022·5	1022·0	1022·5	1023·1
1942 ..	1022·3	1024·2	1024·1	1024·0	1024·3	1023·8	1016·1	1021·2	1024·2	1024·0	1024·0	1023·0

Theoretically speaking, the rise in the specific gravity of the sea, from August to November, may be attributed to the dissolution of the mineral and organic substances emptied by rivers. From August to May there is

considerable sunshine with the result that the temperature rises *pari passu* with the specific gravity. It does not therefore seem unreasonable to conclude that evaporation on account of temperature is also responsible for the parallel rise in specific gravity.

5. Plankton

After qualitative analysis in the laboratory the total quantity of plankton is estimated in c.c. with the help of a sedimenteer and stempel flask. For



Plankton

facilitating the present study the average quantity of each month for the 5 years has been tabulated. As plankton varies from sea to sea, a short account of what occurs in the Arabian Sea, we venture to say may not be out of place. After an examination of a few samples of the plankton, one

is struck by the variety and number of the phytoplanktonic organisms such as *Coscinodiscus, Biddulphia, Fragillaria, Thallassiothrix, Pleurosigma, Nitzschia, Rhizosolenia, Chætoceras,* etc. The long ribbons of *Fragillaria* and *Thallassiothrix* are really striking objects to say nothing of the *Chætoceras* chains. Occasionally the student will be bewildered by a pure culture of that inedible *Cystoflagellate, Noctiluca,* which is suspected to be responsible sometimes for the local scarcity of the oil Sardines and other fishes. Amongst the zoo-plankton, copepods, arrow worms, leucifer, larval crustaceans, fish eggs and larvæ and ctenophores are organisms of common occurrence. In this paper it is proposed to discuss the relationship of the variation of the total quantity of plankton to the fiuctuation of the fisheries in general. The inter-relationships of the different varieties of the organisms in the plankton amongst themselves or their co-relation to the fisheries are so complex that they can form subjects for separate studies.

It is regrettable that during the months of S.W. Monsoon in June, July and August plankton collections are not possible owing to the stormy weather conditions. The volumetric studies are therefore limited to almost nine months in the year.* The volume of plankton is at its maximum in the month of September. In October and November there is a fall with the slight increase in December followed by a subsequent poverty of plankton in the month of January. In the months of February, March, April and May the productivity of plankton is more or less steady with a minor fluctuation occasionally. The richness of plankton in August and September is perhaps due to the combined influence of fairly long hours of sunshine, low temperature and the intermixing of the different strata of water containing rich nutrient salts brought down by the floods. The comparatively high productivity during the hot dry season from February to May is caused by the intense sunlight encouraging greater photosynthetic activity and uniform vertical mixing up of the different layers of waters of the sea.

TABLE IV

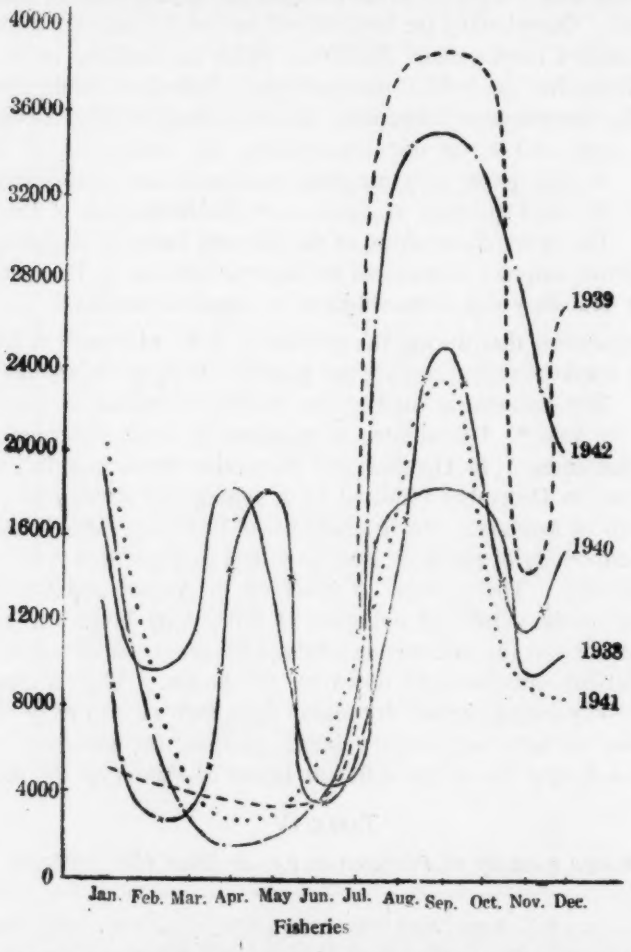
Average quantity of Plankton in c.c. at West Hill, 1938–42

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1938 ..	32·25	40·5	56·77	53·0	86·44	..	..	53·0	36·14	53·5	78·33	58·66
1939 ..	43·82	37·5	46·32	43·22	41·3	..	..	80·2	88·4	38·08	21·0	45·0
1940 ..	26·75	49·0	43·33	43·07	46·15	39·0	..	22·5	80·5	27·22	..	28·33
1941 ..	26·25	37·0	40·0	57·3	52·0	..	..	..	177·5	77·0	48·0	62·45
1942 ..	70·16	63·0	79·17	18·7	69·0	..	98·4	93·1	29·9	41·2	24·0	19·73

* This explains the gap in the graph.

6. Fisheries

The fishing season in the locality extends from August of one year to February of the succeeding year. The average landings amount to 1,50,000



Fisheries

maunds. The fishery reaches its peak in the months of August, September and October and then there is a gradual fall till June when due to the onset of the monsoon fishing is suspended. In July the landings are fairly good averaging about 9,000 mds. Mackerel, Oil sardine, Horse mackerel, Sharks, Soles and Ribbon fish contribute mainly to the catches during the months from September to March. In April and May fishing is restricted mainly

to Prawns, small Soles and occasionally heavy landings of Cat fishes. The minor peak observed in the month of May 1940 is due to the landings of 15,130 mds. of Cat fish which constituted 82% of the catches in that month; similar huge landings of cat fish amounting to 7,175 mds. (70%), 18,185 mds. (98%) and 11,400 mds. (80%) are responsible for the peak in the months of March, April and May in 1938. Silver bellies and Ribbon fish are landed in huge quantities in the months of July and August whereas the fishery of Chamban (*Caranx crumenophthalmus*) extends from August to October.

TABLE V

Landings of fish in maunds at Calicut, 1938–42

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec
1938	16310	9737	10835	18450	14435	4650	5330	17810	11725	18265	9370	10590
1939	5110	5210	3130	5715	5970	3040	7380	38380	10910	38635	14789	27320
1940	12630	3036	2933	3390	18380	3576	8970	12250	25318	12174	11967	26390
1941	21345	6375	6600	4479	3040	4805	13195	13090	23685	16470	9290	8790
1942	18890	7975	3415	1485	4560	3390	9105	33150	35280	24530	22504	2034

7. DISCUSSION

Changes in temperature, rates of evaporation during the different seasons and the rainfall in different months give rise to regular variations in the conditions under which marine fishes live; in Indian waters this variation has been reported to have "a period of approximately 6 months the temperature and salinity of the upper layers of the waters having two maxima in May and September respectively. Again the rotation of the earth and the influence of the winds of the monsoon season give rise to internal 'seiches' that produce oscillating changes in salinity and temperature of the water at definite levels" (R. B. S. Sewell, 1925).

In the sea opposite West-Hill the surface temperature of sea-water exhibits a bimodal curve with a major peak in the month of April and a minor peak in the month of November. During the S.W. Monsoon the temperature falls down to as low as 24·85° C. (in August 1940). The second fall to about 28° C. is in the months of December and January when the effect of N.E. Monsoon is felt slightly on the West Coast. The rise in temperature in the month of April is mainly due to the effect of the sun's rays striking vertically from above and increased biological reactions of the developing planktonic organisms even though there may be a little cooling down owing to the increased evaporation at that time.

Consequent on the increase in temperature, the specific gravity is high at the two different seasons and the temperature also is high. The fall in

Combined Tabular Statement of the Different Factors for the Period 1938–42

	January	February	March	April	May	June	July	August	September	October	November	December
						1938						
Rainfall ..	..	..	·08	9·9185	5·13	30·829	41·245	16·267	6·54	7·233	·44	..
Surface Temperature	27·45	27·95	29·75	29·75	29·8	27·35	25·4	25·55	26·7	27·6	29·5	27·5
Specific Gravity ..	1022·0	1022·0	1021·8	1022·0	1022·0	1022·0	1019·0	1020·0	1021·0	1022·0	1022·3	1022·5
Volume of Plankton	32·25	40·25	56·77	53·0	86·44	..	..	53·0	36·14	53·5	78·33	58·66
Fishery ..	16310·0	9737·0	10835·0	18450·0	14435·0	4650·0	5330·0	17810·0	11725·0	18265·0	9370·0	10590·0
						1939						
Rainfall ..	..	..	..	3·21	·112	28·14	35·68	9·82	3·35	10·624	7·23	..
Surface Temperature	27·35	27·9	28·7	29·95	29·0	26·7	26·25	26·55	25·85	28·5	28·5	27·7
Specific Gravity ..	1023·0	1023·0	1023·0	1021·0	1023·0	1023·0	1018·0	1021·0	1023·0	1022·0	1021·0	1023·0
Volume of Plankton	43·82	37·5	46·32	43·22	41·3	..	..	80·2	88·4	36·08	21·0	45·0
Fishery ..	5110·0	5210·0	3130·0	5715·0	2970·0	3040·0	7380·0	38380·0	10910·0	38635·0	14789·0	27320·0
						1940						
Rainfall ..	..	..	..	·15	8·83	22·47	47·60	20·12	1·73	11·12	4·88	..
Surface Temperature	27·05	28·10	28·45	30·5	30·1	28·15	25·75	24·85	26·33	29·25	29·65	28·9
Specific Gravity ..	1020·9	1022·8	1023·8	1023·0	1023·70	1023·3	1017·5	1020·0	1023·5	1022·5	1022·0	1022·0
Volume of Plankton	26·75	49·0	43·33	43·07	46·16	39·0	..	22·5	80·5	27·22	..	28·33
Fishery ..	12630·0	3036·0	2933·0	3390·0	18380·0	3576·0	8970·0	12255·0	25318·0	12174·0	11967·0	26390·0
						1941						
Rainfall ..	·29	..	..	2·09	20·34	38·14	14·621	16·21	6·65	11·32	8·43	..
Surface Temperature	28·45	28·65	30·25	31·20	29·55	27·30	26·55	26·2	26·75	28·25	29·90	28·80
Specific Gravity ..	1022·5	1023·0	1025·6	1023·4	1023·5	1022·0	1018·0	1021·0	1022·5	1022·0	1022·5	1023·1
Volume of Plankton	26·25	37·0	40·0	57·3	52·0	..	..	..	177·5	77·0	48·0	62·45
Fishery ..	21345·0	6375·0	6600·0	4479·0	3040·0	4805·0	13195·0	13090·0	23685·0	16470	9290·0	8790
						1942						
Rainfall ..	..	..	·14	3·73	6·27	37·44	34·11	10·71	2·91	12·34	2·87	3·28
Surface Temperature	27·90	29·10	30·25	30·50	29·55	27·45	26·2	25·35	25·65	28·10	28·80	28·20
Specific Gravity ..	1022·3	1024·2	1024·1	1024·0	1024·3	1023·8	1016·1	1021·2	1024·2	1024·0	1024·0	1023·0
Volume of Plankton	70·18	63·0	79·17	18·7	69·0	..	98·4	93·1	29·9	41·2	24·0	19·73
Fishery ..	18890·0	7975·0	3415·0	1485·0	4560·0	3390·0	9105·0	33150·0	35280·0	24530·0	32504·0	20344·0

the specific gravity in July is caused by the influx of fresh water from the rivers and from the rains. The relationship between surface temperature and specific gravity is direct. But the rains during the monsoons, when sometimes the average rainfall is about 40" in June, effect the marked changes in specific gravity and surface temperature and again the showers of the N.E. Monsoons cause the second fall in temperature and specific gravity in the month of November.

As indicated above the planktonic organisms are very rich in the months of July, August and September, when the surface temperature and specific gravity are low. The rains which commence late in May or early in June wash into the sea plenty of nutrient salts for enriching " the pasture of the sea". The statement, that " the supplies of nutrients brought up from below by the S.W. Monsoon support a large but short-lived outburst of phyto-plankton which develops in the increasingly stable conditions following the change of wind and subsequently provides food for a rich zoo-plankton ", made by Thompson and Gilson in connection with the South-Eastern section of the Arabian Coast holds equally good in this area. During the later period of the monsoon, *i.e.*, in August and September, the rise in plankton productivity is contributed by the intermingling of the various salts in the upper layers of the sea and the prevailing calm and settled conditions of weather whereas in the earlier period the development of planktonic organisms is arrested partly by the lack of sunshine and partly by the unstable conditions of the different strata of water. There is a gradual fall in the productivity of plankton till November when the supplies of nutrient salts perhaps become exhausted. In December there is a slight rise in the productivity due to lowering in surface temperature, the renewal of the supply of nutrient salts owing to the occasional outbursts of rain caused by the N.E. Monsoon and the upwelling of the waters. In the months of February, March, April and May there is a steady appearance of plankton in the surface waters due to the reappearance of calm weather, stability in surface waters and a steady though not rich supply of nutrient salt from the Bay of Bengal brought by the change in the current system during that period.

The landings of fish is directly proportional to the quantity of plankton thus indicating the direct relationship of plankton as food to fishes. The fishery in general coincides with the major peak in the plankton productivity. The comparatively low catches in the months of March, April and May are due to the increase in temperature, in the inshore waters which result in a temporary migration of fish to the cooler off-shore regions.

8. Summary

1. There are two peaks of rainfall, the major one in July to August due to S.W. Monsoon and the minor one in October effected by N.E. Monsoon.

2. The surface temperature is high in April and low in August followed by a gradual increase up till November when again there is a slight fall.

3. The changes in specific gravity are parallel to the changes in surface temperature.

4. The maximum productivity of plankton takes place in the months of July, August and September followed by a rise in December due to the showers of the N.E. Monsoon and again a period of steady production.

5. The landings of fish are directly proportional to the quantity of plankton produced in the different months of the year. The fishery has invariably been found to be good after heavy rains when they bring plenty of 'indispensable food substances', thus enriching the "pasture of the sea" and increasing the food of fishes.

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FURTHER OBSERVATIONS ON THE STRUCTURE AND FUNCTION OF THE NIDAMENTAL GLANDS OF A FEW ELASMOBRANCHS OF THE MADRAS COAST

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Received October 6, 1945

(Communicated by Dr. N. Kesava Panikkar, F.A.SC.)

Introduction

The structure and various modifications in the nidamental glands of certain ovo-viviparous and viviparous elasmobranchs have been described in another paper (Prasad, 1945). Studies on similar lines have been continued on four other species of elasmobranchs from the Madras Coast in order to find the variations that occur in the structure of these glands among members having the same mode of breeding and as oviparity gives place to viviparity and to see whether these characters have any relationship to the phylogeny of elasmobranchs. As the main object of this study is not the histology of these glands I have only given the salient features in their structure. Moreover, except for the size and shape of the tubules, cells and nuclei there is no difference between the histology of the glands of these and that of other species already described. Observations as to whether the gland acts as a receptaculum seminis have also been made as before.

I take this opportunity to thank Dr. N. Kesava Panikkar, Director, University Zoological Research Laboratory, for his kindness and suggestions.

Galeocerdo tigrinus Mull. and Henle

The nidamental glands of *G. tigrinus*, the tiger shark, are two massive structures one on either side of the vertebral column and both these are equally developed. The average size of the nidamental gland is 199 mm. in width and 108 mm. in length whereas the average size of the parent is about 3,775 mm. The central portion of the nidamental gland gives off two twisted diverticula each placed on either side of the cranial oviduct (Text-Fig. 1). The nidamental glands are covered over by a thick vascular mesometrium and have a uniform flesh colour. The central lumen gives off lateral lumina into the horns which follow the course of the horns and in shape they resemble the lumina of the lateral horns of the nidamental glands of *Scoliodon palasorrah*. The inner surface at the anterior albumen

secreting zone shows shallow grooves and ridges and at the posterior region, *i.e.*, the shell secreting zone—lamellæ.

A longitudinal section passing through the different regions of the nidamental gland shows three regions, *viz.*, the anterior long albumen secreting zone of 20 mm., the middle short cranial shell-secreting region measuring 2 mm., and the posterior caudal shell-secreting zone measuring 5 mm. in length. The albumen secreting tubules, opening between the cranial transverse bands, are almost circular in transverse section and are made up of gland cells and ciliated cells. The lamellar region extends up to the caudal shell-secreting region and thus the tubules of both the shell-secreting regions open between lamellæ. These lamellæ are of almost uniform height with broad free ends. The 'tufts' accompanying them are fairly long and pointed in sections. The epithelium covering these is uniformly ciliated.

Spermatozoa were noticed in the posterior shell-secreting tubules of the nidamental glands of two specimens of which in one specimen each uterus contained 20 eggs and in the other the eggs were seen in the cranial oviduct. Three more specimens of the same species were examined and of these one was post-pregnant and the remaining two were immature; none of these had sperms in the nidamental glands.

Myliobatis nieuhofii (BLOCH AND SCHNEIDER)

The nidamental gland of this species is a mere swelling of the oviduct and there is no indication of the lateral horns (Text-Fig. 2). Only one gland is fully developed. As the right ovary and uterus are not well developed the nidamental gland on that side is also not well developed. The external appearance of the nidamental gland shows two well-defined regions, *viz.*, the anterior pink albumen secreting zone and the posterior yellow shell-secreting zone. The inner aspect shows shallow grooves alternating with ridges at the anterior region and lamellæ-like structures at the posterior region. There is a single central lumen.

A longitudinal section of the nidamental gland of this species shows the two zones already mentioned. The albumen-secreting tubules are arranged as in other forms and open in the region of the cranial transverse bands. The albumen-secreting zone is short and measures only 3 mm. in length. The tubules are built essentially on the same model as those of others with gland cells having round nuclei and the ciliated cells having elongated nuclei. In transverse sections the tubules appear to be round and in size they approximate those of the shell-secreting zone.

The shell-secreting zone forms the major portion of the gland measuring 7 mm. in length and is not divided into the cranial and caudal zones. The secretory activity of these tubules is fairly pronounced and the granules of secretion stain blue-black with Heidenhain's iron-hæmatoxylin. The reactions of the different regions to different stains are given in Table I. The

TABLE I

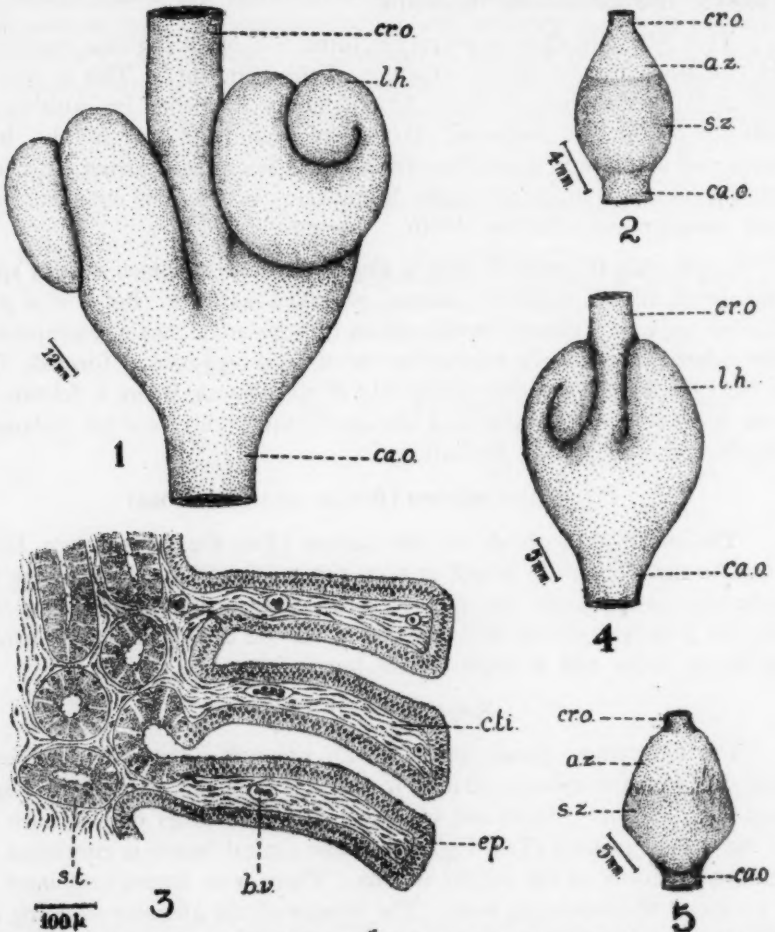
Reaction of the different regions of the nidamenal gland to different stains after fixation in Zenker's fluid

Section of the nidamental gland of	Heidenhain's iron-haematoxylin			Mallory's triple stain			Hoyer's Thionin blue		
	Albumen glands	Shell glands		Albumen glands	Shell glands		Albumen glands	Shell glands	
		Cranial	Caudal		Cranial	Caudal		Cranial	Caudal
G. tigrinus ..	Grey	Black	Blue-black	Light blue with a slight red tint	Bright red	Light red with a blue tint	Light blue	Pale blue	Light blue
M. nieuhofii ..	Pale grey	Blue-black		Light blue	Bright red		Light blue	Pale blue	

tubules open between ' lamellæ ' (Text-Fig. 3) totalling about 31. They are of uniform thickness with flat distal ends and are almost of the same height. An interesting feature is noticed in this species. The ' lamellæ ' are not accompanied by ' tufts' which are so characteristic of other forms and these are different from the cranial transverse bands only in their size. Tbe epithelium covering these ' lamellæ ' is of uniform thickness whereas in other species already studied by me the epithelium of the lamellæ consists of cubical cells having round nuclei at the base and elongated cells with almost oval nuclei at the distal end. In this species the basal cells as well as those at the distal end are of the same thickness; they are similar in being rectangular with oval nuclei and in being uniformly ciliated.

In all pregnant examples of other species examined the uteri showed remnants of egg-cases, however much advanced the stage of pregnancy. But in this species even though the uterus contained embryos measuring 270 mm. (including tail), there was no trace of egg-case in the uterus. It may be pointed out that an examination of even a post-pregnant uterus may often reveal the torn remnants of egg-cases. It has already been stated that in this species the shell-secreting tubules open between mere folds of epithelium devoid of ' tufts '. It is probable that the function of these ' tufts ' is to

guard the opening of the tubules and help in moulding the egg-case. Probably the absence of these 'tufts', which are of importance in the proper



Text-Figs. 1–5.—Fig. 1. The nidamental gland of *G. tigrinus*. Fig. 2. The nidamental gland of *M. nieuhofii*. Fig. 3. Section showing the 'lamellæ' in the shell secreting region of the nidamental gland of *M. nieuhofii*. Note the absence of 'tufts'. Fig. 4. The nidamental gland of *S. blochii*. Fig. 5. The nidamental gland of *P. micrura*. *a.z.*, Ablumen secreting zone; *b.v.*, Blood-vessel; *c.ti.*, Connective tissue; *ca.o.*, Caudal oviduct; *cr.o.*, Cranial oviduct; *ep.*, Epithelium; *l.h.*, Lateral horn; *s.t.*, Shell secreting tubule; *s.z.*, Shell secreting zone.

formation of the egg-case, indicates that no egg-case is formed. It is likely that the shell secretion is poured into the uterus and this along with the albumen may form a sort of nutrient material. In this connection the

observation of Couch (1847) is noteworthy as he has observed the egg-case in an undetermined species of *Myliobatus* and remarks that the egg-case he observed had the surface reticulated.

That probably there is no proper formation of an egg-case may further be confirmed by the simple structure of the 'lamellæ'. This is not only indicated by the absence of the 'tufts' in the 'lamellæ' but also by their uniform height and thickness. It may be mentioned that in the highly developed nidamental glands like those of *Chiloscyllium griseum* the delicate lamellæ show variations in shape, height, etc., between the anterior, middle and posterior ones (Nalini, 1940).

I was able to examine only a single pregnant specimen of this species and hence the conclusion cannot be taken as final. But if it is a fact that no egg-case is formed in this species it forms an interesting example where the nidamental gland is present but no definite egg-case is formed. Thus it will take a place between species like *S. sorrakowah*, where a delicate egg-case is formed, and *Narcine* and *Astrape* in which there are no nidamental glands and no egg-case formation.

Pteroplatea micrura (BLOCH AND SCHNEIDER)

The nidamental glands of this species (Text-Fig. 5) measure 12 mm. in width and 15 mm. in length in a specimen of 809 mm. (including tail). Externally two regions, the anterior flesh coloured albumen-secreting zone and the posterior yellow shell-secreting zone, are distinguishable. There are no lateral horns and a single central lumen is present.

Sphyrna blochii (CUV.)

The nidamental glands of *S. blochii* are well developed, uniformly flesh coloured and measure 22 mm. in length and 20 mm. in width in a specimen of 1525 mm. There are two twisted lateral horns one on each side of the cranial oviduct (Text-Fig. 4) and the central lumen is continued into the lateral horns as the lateral lumina. There is an anterior albumen and a posterior shell-secreting zone. The tubules of the albumen secreting zone open between cranial transverse bands while those of the shell-secreting region open between lamellæ.

SUMMARY

The structure of the nidamental glands of *G. tigrinus*, *M. nieuhofii*, *S. blochii* and *P. micrura* is described.

Spermatozoa have been observed in the shell-secreting tubules of the nidamental gland of *G. tigrinus*.

'Tufts' which are characteristic of the lamellæ of other nidamental glands, are absent in the 'lamellæ' of the shell-secreting region of the nidamental gland of *M. nieuhofii*. No remnant of egg-case is seen in the uterus and it is suggested that owing to the absence of the 'tufts' and the simple structure of the 'lamellæ' probably no egg-case is formed in this species.

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AN ASCOMYCETOUS PARASITE OF *CEPHALEUROS*

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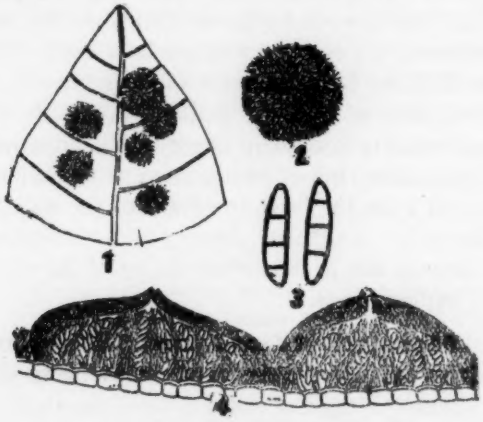
Received October 31, 1945

(Communicated by Prof. L. Narayan Rao, Ph.D., F.A.Sc.)

Species of *Cephaleuros* are well known algal parasites on the leaves and tender shoots of many economic plants. *Cephaleuros mycoidea* Karst. commonly found distributed on various hosts is regarded as a very superficial parasite. On the other hand *Cephaleuros parasiticus* Karst. according to Petch (1923) and others, is a very serious parasite on account of the fact the algal filaments penetrate the epidermis and spread within the mesophyll tissue. The red rust disease of tea is caused by this species and the damage is particularly significant when the shoots are parasitised. The heavy infestation of the parasite results in the production of numerous necrotic spots causing severe defoliation of the shoots.

Both *Cephaleuros parasiticus* and *C. mycoidea* have been collected on a large number of hosts in Mysore and particularly the former causes fruit blemishes in *Psidium Suajava*. In most of the collections the algal patches appeared to have been killed by an ascomycetous fungus the entire patch being studded with the fructifications of the fungus (Figs. 1 and 2). In a healthy patch the thallus is discoid with large number of erect branching filaments. Laterally borne stalked sporangia are produced in profusion. The contents of the cells in the entire thallus are orange red on account of the presence of hæmatochrome which masks the green colour of the chloroplast. But after the parasitisation of the algal filaments by the fungi, the growth along the margin of the radiating filaments ceases, the entire patch becoming gradually crustose and white in colour. The erect branching trichomes and the stalked sporangia which impart a hairy appearance to the thallus can no longer be observed in the infected patch. This difference can be very clearly noticed in the case of a partially infected patch when we can notice that the reproductive structures of the alga are no longer produced after the invasion by the fungus. Microscopic examination of the algal filaments from such areas reveal that they are completely devoid of the characteristic orange red contents and are in fact killed by the fungus. In later stages, the development of the fructifications of the fungus can be observed throughout the crustose patch.

The perithecia are black, minute and semiglobose appearing prominently on the surface as black specks. There is a small ostiolar pore at the apex, which in some cases appears to be situated on a slight elevation over the wall of the perithecium. The asci (Fig. 4) are numerous, clavate-cylindric, paraphysate, the paraphyses being free and persistent and not becoming mucilagenous at maturity. The ascus is 8-spored, the spores being long and spindle shaped, 4-septate and hyaline (Fig. 3). Small groups of spermogonia with hyaline spores have been noticed occassionally.



Figs. 1–4.—Fig. 1. Algal patches of *Cephaleuros* on leaves of *Dubanga* sp. nat. size. Fig. 2. Enlarged view of the parasitised thallus showing perithecia. ×3. Fig. 3. Ascospores ×1800. Fig. 4. Section through the perithecia. ×200.

Study of literature indicates that the form under study is identical with the ascolichen genus *Strigula* E. Fr. and comes very near the species *S. astridiza* Vain. recorded from the Philippines. The genus *Strigula* included under the Verrucariaceæ by Zahlbruckner (1926), is stated to be formed by the association of algæ like *Cephaleuros, Phyllactidium* and *Heterothallus* with an ascomycetous fungus. The genus was founded as far back as 1821, and according to Zahlbruckner includes about 25 species.

The true nature of the relationship of the algal and fungal components within a lichen has been much debated without any final opinion. At least in those cases wherein there is some sort of symbiotic relationship usually termed as helotism, the alga and the fungi live in mutual association. But the work of Fry (1928) working on the subject of the penetration of the gonidia by the fungal constituent in *Lecania candicans* and other workers indicate the true parasitic nature of the fungus. The development of the haustoria within the gonidial cells by the fungus resulting in the gradual

depletion of the cell contents and the death of the algal cells confirms the parasitic nature of the fungal component. It is for this reason that workers like Bessey (1935) and Clements and Shear (1931) consider the lichens as fungi parasitic on the algal substrata during all or part of their life-cycle. They hold the view that the algal component might be neglected, and according to the nature of the fungus they are grouped in Ascomycetes or Basidiomycetes.

The species of *Strigula* under study appears to furnish a very suitable example for the point of view that the fungus is primarily a parasite. In this connection the observations by Butler (1918) on the same subject with reference to *Cephaleuros* on tea plants might be quoted, " The death of the alga is sometimes hastened by an invasion of fungal hyphæ which are found very frequently intermingled with the thallus. Some observers have considered that lichen formation is brought about by this invasion, the two organisms living in association, but it seems more probable that the fungus is wholly injurious, and from the first, an enemy of the alga ". The observations made by the writer completely support Butler's findings, the crustose lichen-like appearance is due to the cementing up of the dead algal filaments by the gelatinous hyphal masss.

In the case of lichens such as *Lecania candicans* the gonidial cells are no doubt parasitised by the fungus, the empty cells being discarded during exfoliation. On account of rapid multiplication of the algal cells and possibly by the weak parasitisation by the fungus, the algal component continues to be present within the lichen thallus. As against this, the filaments of the *Cephaleuros* thallus are completely killed by the invasion of the fungus and it is very doubtful whether the alga could be regenerated at all from such an infected patch. *Strigula* thus presents an extreme type in which the algal component does not exist as a living partner but only serves as a source of nutrition for the parasitic fungus; thereby lending support to the views of Bessey and Clements and Shear.

In the description of the genus *Strigula*, Zahlbruckner (1926) refers to the fructification of the fungus as an apothecium characteristic of the Discolichens though in the illustration it is presented as a perithecium (probably taken along with the original figure). Clements and Shear (1931) include *Strigula* and all the members classed under Verrucariaceæ of Zahlbruckner in the Sphæriales with perithecia and ostiole. While the presence of perithecium and ostiole are correctly described by Smith (1911), and Clements and Shear, the semiglobose nature of the perithecium simulating the upper portion of a perithecium and other characters indicate that it could more properly be classed under Hemisphæriales (Microthyriaceæ of Clements and Shear).

The methods of control of the red rust disease on tea as practised in India and the measure of success by the application of each method have been described in detail by Butler (1918). The possibility of the disease, which also occurs on the jungle trees, being blown over on to the tea plants cannot be overlooked. Improving the vigor of the tea bushes by cultural practices and the raising of new estates from plants showing a high degree of resistance to red rust, are indirect but very important methods of reducing the prevalence of the disease. The difficulty of wetting the algal patches when they are in fruiting has been pointed out by Butler, as rendering the Bordeaux misture spraying quite ineffective. In concentrating all our efforts in checking the red rust disease of tea and other plants by such methods as pruning off diseased twigs, spraying with Bordeaux mixture, selection of reistant varieties, etc., the possibility of utilising the hyperparasite as a means of controlling the disease biologically, might deserve consideration.

In conclusion, the writer wishes to express his grateful thanks to Dr. L. N. Rao, Professor of Botany, University of Mysore for his kind encouragement, and to the University of Mysore for the award of the Fellowship.

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ON RANDHAWA'S "A CRITICAL REVIEW OF SOME RECENTLY CREATED NEW SPECIES OF INDIAN ZYGNEMALES"

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The author has read with interest Randhawa's so-called critical review of some recently created new species of Indian Zygnemales, published lately in this Journal (Vol. XVIII, Sec. B, No. 4, 1943). The various remarks made by him, in regard to the new species described by Rao (1937), Misra (1937), Dixit (1937) and the present author (1938) require careful scrutiny and cannot be let off unchallenged. Randhawa has published some papers on the Zygnamales comprising a number of new species and varieties and also two new genera. It is, perhaps, on the basis of this experience that he has endeavoured to criticise some of the new plants described by us and brought out discrepancies which, as will be evident in the following pages, have no sound bearing. Although the writer himself had felt impelled on many occasions to write a critical note on Randhawa's growing list of new genera and species, he could not do so on account of some more important work on algae being in hand. A review of his new plants also would not therefore be out of place here.

A. A careful consideration of Randhawa's Criticism of some New Species of Indian Zygnemales

Genus *Zygnema* Agardh

1. *Zygnema gangeticum* Rao (1937, p. 270)—Randhawa remarks that Rao did not come across mature zygospores because the author describes their mesospore as *thin, smooth and yellowish-brown*. The local alga has been collected from the same locality by different persons at different times year after year and the mesospore has been found to show exactly the same characters as originally described by Rao. The validity of the species therefore cannot be doubted. It may be pointed out that a similar nature of the zygospore wall formed the basis for creating the new species, *Zygema lævisporum*, by Jao (Jao, 1935, p. 5). The same is the case in Randhawa's (1938, p. 145) own new species, *Zygnema giganteum* Randh., where a *thin, light blue and smooth mesospore* has been described in anisogamous conjugation, as

also in his *Z. Czurdæ* Randh. (1936, p. 240). In a previous communication, Randhawa (1938, p. 141) writes that " a form described as *Z. gangeticum* sp. nov. by Rao is presumably a laterally reproducing form of this species " (*Z. Heydrichii* Schmidle). It is strange that *Z. gangeticum* should be described by Randhawa as a laterally reproducing form of *Z. Heydrichii* when the latter alga reproduces itself exclusively by lateral conjugation. Again *Z. gangeticum* is a species with a smooth mesospore, while in *Z. Heydrichii* the mesospore is clearly scrobiculate.

2. *Zygnema indica*, 3. *Z. sphærica*, and 4. *Z. kashmirensis* described as new by Misra (Misra, 1937, pp. 110–2), have again been collected from Kashmir and have been found to show exactly the same characters of the mesospore-wall, even in perfectly mature zygospores. The species in question, therefore, are quite distinct and valid. It is not only on the nature of the zygospore-wall that these species have been established, but on other characters also. *Z. indica* is quite distinct in the section " Pectinata " of the genus *Zygnema*, on account of its ellipsoidal zygospores with a smooth mesospore in which feature it clearly differs from *Z. cyanosporum* Cleve and *Z. majus* Czurda. With regard to *Z. sphærica*, the critic ought to have referred to Misra's foot-note on p. 111 of his paper (Misra, 1937, p. 111). Again in connection with these species Randhawa remarks, " However, the figures of the author clearly show that he was sketching immature zygospores which show chloroplasts". But, similar zygospores with chloroplasts have been sketched by Randhawa himself in *Z. Czurdæ* Randh. and *Z. giganteum* Randh. (*cf.* Randhawa, 1936, Pl. XI, Figs. 4–6, and Pl. XII, Fig. 16 respectively). It may be noted that it is not always necessary to get perfectly mature zygospores. The zygospore wall starts maturation from the periphery and gradually proceeds towards the centre. If the mesospore is even slightly differentiated it is possible to make out its nature. In Misra's figures of his new species such a differentiation has already been completed and therefore the criticism of Randhawa does not hold. Another point raised by the critic is that " too much reliance was placed on the keys of Czurda, which have become out of date, as a number of new species have been described since then from U.S.A., China and India ". Of course, as a guide, we do consult and rely on Czurda's keys, since no other standard work on Zygnemales has yet been published where species described from all places could be found. He should know that before establishing a new species, the plant in question is compared with all other recently described species also.

5. *Zygnema gorakhporense* Singh (1938, p. 370).—In this alga Randhawa points out that "the spore-wall is described as *lamellate with broad scrombiculations* but in the figures the *pits* are not shown ". It is really very surprising

that the critic has failed to make out the pits from the Text-Fig. 1, A and B (*cf.* Singh, 1938, p. 371), although the study of the surface of a rounded structure under the microscope is so very easy to follow. The zygospore in this species is a globose to subglobose structure and can be seen under the microscope in so many different views. The view sketched is the one where only part of the circumference of the pits could be seen. Before making the hasty remark mentioned above, Mr. Randhawa should have referred to *Z. compressum* (p. 116, Fig. 119, *c*), *Z. Hansmannii* (p. 121, Fig. 125, *c*), and *Z. germanicum* (p. 125, Fig. 129, *a*), described by Czurda in " Pascher's Süsswasser-flora, Mitteleuropas, Heft 9, 1932 ", where a similar representation of the pits has been made. This is just to quote a few cases in the genus *Zygnema* itself, leaving aside the other genera where many more such examples can be found.

Genus *Spirogyra* Link.

1. *Spirogyra azygospora* Singh (1938, p. 372).—In this case Randhawa's remarks are, " While Singh has described the mode of reproduction as by means of azygospores, he actually gives the length and breadth of zygospores. This is probably a mistake." The omission of the letter 'a' before the word ' zygosp.' in the second paragraph, is a press omission which was perhaps overlooked during proof-reading. But, in the description and discussion given in the first and third papragraphs the word ' azygospore ' is however quite distinct. Moreover the relevant figure shows the azygospores quite distinctly. With regard to the validity of this species Randhawa's further remarks are, " Formation of azygospores is, however, not a permanent feature in any member of the Zygnemales, as due to interruption of normal conjugation azygospores may be formed in any species which normally produces zygospores. The production of azygospores is only a physiological feature and is not of any morphological and systematic importance. In dimensions of cells and number of chloroplasts Singh's *S. azygospora* resembles *S. submaxima* Transeau." *S. azygospora* Singh has been established on the authority of Czurda. It is valid to the same extent as other few azygosporic species, such as, *S. mirabilis* (Hassall) Kuetz. (1849), *S. Oltmannsi* Huber-Pestalozzi (1930), and *S. Narcissiana* Transeau (1914)—described by the world authority Czurda (1932), and, also *S. articulata* Transeau (1934) described by Transeau, Tiffany, Taft and Li (1934), where reproduction is exclusively by azygospores. It is a fundamental mistake to include all such species under the same category, where besides the formation of azygospores other modes of reproduction are also known. In the latter cases the azygospores are expected to be formed by the interruption of normal reproduction due to internal physiological causes. But, such species as

produce only azygospores at all times make a category of their own and should not be treated as physiological forms. It was on this basis of the exclusive formation of azygospores that Czurda (1932, p. 131) created a separate section " Mirabiles " in the genus *Spirogyra* to include these species. If Randhawa believes that the formation of azygospores in all the Zygnemales cannot be taken as a permanent feature and is only due to the interruption of normal process of conjugation, it is surprising that he should have created so recently a new species, *S. aplanosporum* Randh. (*cf.* Randhawa, 1938, pp. 336–7). This species as will be evident later in this communication, does not appear to be a valid one on several grounds.

2. *Spirogyra Czurdæ* Misra (1937, p. 115).—According to Randhawa, *S. Czurdæ* is actually *S. luteospora* Czurda, but it is wrong. The former is a distinct species with perfectly spherical zygospores whereas in *S. luteospora* Czurda the zygospores are clearly " langachsig-ellipsoidisch ".

3. *Spirogyra paradoxa* Rao (1937, pp. 281–2).—Before making a criticism of Rao's *S. paradoxa*, Randhawa should have referred to the foot-note given by Rao on p. 282 in his paper (Rao, 1937), where it has been mentioned that the description of *S. setiformis* as given by Czurda (*cf.* Czurda, 1932) is not complete. *S. paradoxa*, according to Randhawa, is an attenuated form of *S. setiformis* (Roth) Kuetz., but it is not correct, as the former species possesses characteristic fructifying cells, that are commonly swollen on the conjugating side, together with spherical zygospores, narrower but almost quadratic vegetative cells, and also lesser number of chloroplasts. It may be noted that it is on the basis of the presence or absence of inflation of the fructifying cells that Jao (1935) established *S. gibberosa* Jao and *S. fragilis* Jao, and Transeau, Tiffany and others (1934) founded *S. supervarians* Trans., in which the receptive gametangia are inflated on the inner side only.

With regard to *Spirogyra crenulata*, *S. kundænsis* and *S. Ghosei* (not *Ghoseli* as Randhawa has given), described as new by the author (*cf.* Singh, 1938), it may be pointed out that these species possess unique mesospore sculpturing which the author has been observing in collections made year after year since the time they were first described. They cannot be confused with any of the species of *Spirogyra* so far described.

Genus *Sirogonium* Kuetz.

Sirogonium indicum Singh (1938, p. 384).—Randhawa remarks that " Singh describes the spore-wall of *S. indicum* as *irregularly scrobiculate* which is obviously wrong since it cannot be scrobiculate when it is irregular." It is very strange that Randhawa having described so many

species of the Zygnemales with scrobiculate mesospore does not understand the definition of the same. His criticism that "it cannot be scrobiculate when it is irregular" is absolutely wrong; and it is a fact that scrobiculate markings are also irregular. In this connection he should refer to Jao's description of *Mougeotia globulispora* Jao, where he describes the mesospore as "mesosporio subtiliter et *irregulariter scrobiculate* vel reticulato" (*cf.* Jao, 1936, p. 60). Randhawa further writes that "A careful perusal of the above figures (meaning thereby his tabular statement showing a comparison of *S. ventersicum* Trans., *S. inflata* Dixit, *S. indicum* Singh and *S. ventersicum* Trans. var. *melanosporum* Randh.) will clearly show that the so-called *S. inflata* Dixit and *S. indicum* Singh differ only in minor details from *S. ventersicum* Transeau"; and later on that "The present author is of the opinion that *S. inflata* Dixit and *S. indicum* Singh are not valid species and the samples described are covered by the descriptions of the present author's description of *S. ventersicum* Tran. var. *melanosporum*." The present author has also described a variable form of *S. ventersicum* Trans. in his paper on "The Zygnemoideæ of the United Provinces, India—II", along with *S. indicum* Singh. Even a casual observation of his figures of these two species on p. 283 will clearly bring out the difference between the mesospore markings of the two. Randhawa has failed to identify a verrucose mesospore while describing his new variety, *S. ventersicum* Trans. var. *melanosporum* Randh. (*cf.* Randhawa, 1938, p. 364). The mesospore which he describes as verrucose is not verrucose; but irregularly scrobiculate which the present author has described in *S. indicum* Singh. It may be pointed out that Randhawa, in an earlier publication (1938) in which he describes the various forms of mesospore markings or sculpturings by copying most of the figures of Jao (1935, p. 561), neither defines verrucose mesospore nor has given any figure for the same (*cf.* Randhawa, 1938, p. 119, Fig. 1 and p. 120). *Sirogonium indicum* Singh, which is based on a special type of mesospore marking, *i.e.*, irregularly scrobiculate, is therefore a distinct species which cannot be compared with *S. ventersicum* Trans. and *S. inflata* Dixit. On the other hand, the so-called *S. ventersicum* Trans. var. *melanosporum* Randh., which clearly shows irregularly scrobiculate mesospore besides its other characters being covered up by the description given for *S. indicum* Singh, should be treated as *S. indicum* Singh. Again, *S. inflata* Dixit is quite a distinct sepcies from *S. ventersicum* Trans., from which it differs in the fixed number of chloroplasts, *i.e.*, ten, and very much inflated fructifying cells, besides the differences in the dimensions of the vegetative cells and the zygospores. *S. indicum* Singh and *S. inflata* Dixit are therefore quite valid species and the criticism given by Randhawa (1943) regarding these species is baseless.

Further, Randhawa refers to the black pigment of the zygospores which he has seen in var. *melanosporum* of *S. ventersicum*. In this connection it may be pointed out that it has been confirmed by repeated observations that almost all species of the genus *Sirogonium* show black pigmentation of the zygospores and the colour persists even after preserving the material in formalin for more than 10 years. Dixit and the present author have not failed to observe the black colour in their material but they, like Czurda (1932), and Transeau, Tiffany and others (1934), have considered it unnecessary to mention such a common and regular feature of the zygospores in the species of the genus *Sirogonium*. Moreover, this remark of Randhawa appears to be without any meaning when considered in connection with the discussion on the validity of the species in question.

B. A CRITICAL STUDY OF RANDHAWA'S NEW PLANTS OF INDIAN ZYGNEMALES

Genus *Sirocladium* Randhawa

Randhawa (1941) describes this plant as a new member of the Zygnemales having collected it from the Kumaon Hills, at an altitude of about 5,500 ft. It was found on soft clay under an overhanging rock, at a distance of about 10 ft. from a small waterfall, growing in the form of dark green feltlike radial patches. The moist clay on which it was growing received a light spray from the water fall and was shaded in such a way that it never received direct sunlight.

A slide and a microphotograph of this material was shown to the author for opinion through a private source before its account was actually published. The writer then explained to Mr. Randhawa through the same source that the alga in question was nothing else but an ordinary *Sirogonium* possessing rhizoids, in almost drying and degenerating condition. In spite of this, it was surprising to note that the plant came out as a new genus, under the name of *Sirocladium*. Randhawa has based this genus on the following characters:—(1) Terrestrial habit, (2) number of chloroplasts,—only two, (3) nature of chloroplasts, lateral-plates, and (4) mode of conjugation,—*Sirogonium*-like. Let us now examine these characters carefully and see whether they are important enough to warrant the establishment of a new genus.

Regarding the terrestrial habit of the plant it may be pointed out that almost all members of the Zygnemales, although free-floating for the most part of their life-cycle, become terrestrial when ponds, pools and small ditches get suddenly dried up after the close of the monsoon. It appears from Randhawa's description of the habitat of the alga, referred to above,

that the plant in question has also been subjected to a similar circumstance, besides being shaded from direct sunlight. It is for want of the recognition of the consequences of these two adverse ecological factors on the plant which has led Randhawa to create this new genus. When water in situations like those mentioned above dries up, the free-floating plants ultimately come in contact with the soil or clay and as soon as they do so they begin to form rhizoids, probably due to contact stimulus (*cf.* Delf, 1913; and Iyengar, 1927), but most probably due to physiological requirements. Rhizoids have been by now reported by various workers in the genera *Mougeotia, Spirogyra, Zygnema* and *Zygogonium* of the Zygnemales. Their presence, therefore, in the so-called genus *Sirocladium*, should not be surprising. It has often been observed that the chloroplasts degenerate in the cells from which rhizoids develop and so also in the neighbouring cells; and in many cases in the genera *Spirogyra* and *Zygnema* they become plate-like. Such a degeneration of the chloroplasts also takes place when the filaments suddenly get dried up or buried down in the mud, or when they are shaded or cut off from direct sunlight. During the period of conjugation a similar condition also occurs in the neighbouring cells which do not take part in reproduction, as is generally the case in the genus *Sirogonium*. Randhawa's figures 4 and 6 (right-hand portion) clearly show such a degeneration of chloroplasts during conjugation, ultimately bringing their appearance to that of a plate in which the pyrenoids are irregularly distributed. Again in figures 1 and 13–15, regarding *Sirocladium*, most of the cells show a similar degeneration of chloroplasts. The cells which have escaped degeneration possess the two band-shaped (*Sirogonium*-like) chloroplasts (*cf.* also Fig. 7). The latter have been described by Randhawa as thread-like and reduced, although they represent the true and real nature of the chloroplasts in this plant. The so-called plate-like chloroplasts in *Sirocladium* which Randhawa describes sometimes as axile (p. 192) and sometimes as lateral (p. 196), on which special stress has been laid for being taken up as the most important character in establishing this new genus, are therefore really due to degeneration. In spite of the fact that Randhawa realized that " it is true that the reduced chloroplasts in some of the subterranean cells show resemblances with some species of *Sirogonium*, in which the number of chloroplasts is few ", even then he describes the chloroplasts of *Sirocladium* as plate-like. Regarding the number of chloroplasts he adds that " there is no known species of *Sirogonium* in which the number of chloroplasts is reduced to the fixed number of two ", and further that " In nearly all the species of *Sirogonium* the number of chloroplasts is usually 5–8 or more, and only in a few cells are they reduced to one or two on account of certain physiological features." Before making such a hasty

statement Randhawa should have referred to the number of chloroplasts in *Sirogonium sticticum* (Engl. Bot.) Kuetz. where according to Czurda (1932) there are " (2-) 3 (-5) chromatophoren " and according to Jao (1935) there are 3–4 chromatophores. Further, in var. *megasporum* Jao, Jao (1935) records 3–4 chromatophores, sometimes 2, straight or making 0·5 turns in the cell. Randhawa has also mentioned the varied nature of the chloroplast (p. 196). This fact and his observation that the so-called two chloroplasts sometimes cover up each other in such a way as to appear as one (p. 192) should have been a sufficient ground for him to doubt his own interpretations. A more thorough examination of the material of *Sirocladium* is likely to reveal the same variability in the number of chloroplasts as found in *Sirogonium sticticum* (Engl. Bot.) Kuetz. The two genera being the same is further substantiated by the exactly similar modes of conjugation, even in minute details.

In the light of the correct interpretation of facts, referred to above, it appears that the plant described by Randhawa is nothing but an ordinary *Sirogonium* with which it does not materially differ, excepting for the terrestrial habit and the presence of rhizoids, characters which cannot be given so much importance as to warrant the establishment of a new genus. The number and nature of chloroplasts (wrongly interpreted by Randhawa) and the process of conjugation are similar in both the genera. Having seen that the alga in question is a *Sirogonium*, let us now examine its specific position in the genus. A comparison with the species of *Sirogonium* so far described shows that the alga in question comes nearest to *Sirogonium sticticum* (Engl. Bot.) Kuetz. as the following comparative table clearly shows.

It is thus evident that *Sirocladium kumaænsis* Randh., except in minor respects, resembles to a very great extent *Sirogonium sticticum* (Engl. Bot.) Kuetz., a well-established species of a well-founded genus. It is really very strange that a plant which did not materially differ from an already known species should have been raised to a generic rank, and it is all the more surprising that it does not even deserve a specific status. In the author's opinion, on the face of the various evidences, Randhawa's plant appears to be a new variety of *S. sticticum* (Engl. Bot.) Kuetz. to be named as variety *terrestris* var. nov. There is no doubt that it is creditable for Randhawa to have recorded the formation of rhizoids in the genus *Sirogonium* and also that of aplanospores. With regard to the latter it may, however, be noted that Prof. Bharadwaja has also collected a species of *Sirogonium* from Kashmir showing reproduction exclusively by aplanospores.

TABLE I

Showing a comparison of Randhawa's Sirocladium kumaoensis *with* Sirogonium sticticum (*Engl. Bot.*) *Kuetz.*

Sl. No.	Characters	*Sirocladium kumaoensis* Randh.	*Sirogoninm sticticum* (Engl. Bot.) Kuetz.
1	Size of Vegetative cells (in microns)	45–64 × 120–210	38–42 × breit (Czurda, 1932) 38–45 × 92–208 (Jao, 1935)
2	Nature of Chloroplasts	Band-like	Band-like
3	No. of Chloroplasts	2 (?)	(2–) 3 (–5) (Czurda, 1932) 3–4 (Jao, 1935) and also 2, in var *megasporum* Jao, Jao,1935)
4	Zygospores :— Size (in microns) Shape Mesospore	 42–70 × 100–118 Ellipsoidal Smooth	 55 × 95 (Czurda, 1932) 48 × 58 × 92–118 (Jao, 1935) Ellipsoidal Smooth
5	Habit	Terrestrial	Aquatic
6	Aplanospores	Present	Absent
	Nov. Nom.	*Sirogonium sticticum* (Engl. Bot.) Kutz. var. *terrestris* var. nov.	

Genus *Ghosella* Randh.

This plant was established as a new genus of the Zygnemales by Randhawa in 1934 (*cf.* Randhawa,1934). While discussing its affinities, he writes that "In 1918, Dr. Fritsch described from South Africa a form which he called *Zygnema* (*Zygogonium*) *spirale*, and which resembles the alga described here in many important features of conjugation and cell details." This fact, together with a careful consideration of the characteristic features (described below), on which Randhawa has based this new genus, will clearly show that the alga in question is a *Zygnema*. In spite of his naming *Zygnema spirale* Fritsch as *Ghosella spirale* (Fritsch) Randh., Czurda (1937, p. 116) in his recent work, "Conjugatæ", considers it to be *Zygnema spirale* Fritsch. Previously Transeau (1925) had considered *Z. spirale* as *Debarya spiralis* (Fritsch) Trans. and later Transeau, Tiffany and others (1934) as *Zygnemopsis spiralis* (Fritsch) Trans. Czurda (1937) does not believe in the transference of *Zygnema spirale* to the genus *Debarya* or *Zygnemopsis*. It thus appears from the above consideration that Czurda, a great authority on the Zygnemales, does not recognize the genus *Ghosella* Randh., which Randhawa (1937, p. 298) later on considers to be a synonym of the genus *Zygnemopsis* Trans. The following are the characteristic features of *Ghosella* according to Randhawa: (1) Chloroplasts,—*Zygnema*-like; (2) complex membrane

thickenings in the conjugating cells as in *Debarya Hardyii* G. S. West; (3) zygospores extending into both the gametangia as in *Debarya africana*; (4) azygospores as in Zygnema fertile Fritsch; (5) zygospores of peculiar shape resembling those of *Debarya desmidioides* and *D. africana* in external appearance, etc.; (6) peculiar dissociation of zygospores is the unique feature of the alga, etc.; (7) the nearly geniculate type of conjugation as in *Mougeotia*; and (8) rudimentary anisogamy in certain cases. Leaving aside the nature of chloroplasts, which Randhawa himself has described as *Zygnema*-like, and the formation of azygospores as in *Zygnema fertile* Fritsch, let us now examine the remaining characters and see whether they are important enough to warrant the establishment of this new genus. Regarding the complex membrane thickenings in the conjugating cells, which Randhawa mentions as one of the diagnostic features of *Ghosella*, it may be pointed out that such thickenings have also been reported in *Zygnema americanum* (Trans.) Czurda, *Z. decussatum* (Trans.) Czurda, *Z. spirale* Fritsch, and *Z. fertile* Fritsch, etc. (Czurda, 1932 and 1937.) Rudimentary anisogamous reproduction has been shown to be a very unstable character by Fritsch and Rich (1927), which will be referred to and discussed in detail later on in this communication in connection with the species of *Zygnema*. Similarly the shape of the zygospores, which is a very variable character, cannot be given much emphasis. Again, the geniculate type of reproduction is not a distinct character since it is known in many species of *Zygnema*, having reported by even Randhawa himself in *Zygnema Czurdæ* Randh. (*cf.* Randhawa, 1936). From the above considerations it is evident therefore that *Ghosella* is invalid and so does not deserve to be a new genus.

Genus *Spirogyra* Link

1. *Spirogyra aplanosporum* Randh. (Randhawa, 1938, pp. 336–7).—According to Randhawa the reproduction in this species takes place exclusively by means of aplanospores, but even then he compares it with *S. subpapulata* Jao, where scalariform conjugation is also known. Such a superfluous attempt could not have been made by an experienced worker in the matter of research, for Czurda (1932) has created a separate section "Mirabiles" in his key to the various species of *Spirogyra* where species which reproduce exclusively by aplanospores are included. Randhawa should have compared this plant with some of the species given under section "Mirabiles". A comparison with the species under this section shows that the alga in question comes close to *Spirogyra mirabilis* (Hassall) Kuetz (1849). In fact, the former appears to be similar to the latter as the following comparative table clearly shows.

TABLE II

Showing a comparative study of Spirogyra aplanosporum *Randh. and* Spirogyra mirabilis (*Hassall*) *Kuetz.*

Sl. No.	Characters	*S. aplanosporum* Randh. (1938)	*S. mirabilis* (Hassall) Kuetz. (1849)
1	Size of vegetative cells (in microns)	20–26 (breadth)	24–26 (breadth)
2	Nature of septa	Plain	Plain
3	No. of chloroplasts	Single	Single
4	Shape of fructifying cells	Swollen, almost globose	Swollen
5	Reproduction	By aplanospores	By aplanospores
6	Aplanospores :—		
	Size (in microns)	24–30 × 30–54	30 × 35 ; 23 × 30
	Shape	Globose to ellipsoidal	Globose to long-ellipsoidal
	Mesospore	Smooth	Smooth with a ribline

2. *Spirogyra Sahnii* Randh. (Randhawa, 1938, pp. 339–41).—While discussing the affinities of this alga Randhawa writes that "From *S. condensata* (Vauch.) Czurda emend., it differs in the shape and size of vegetative cells, the size of zygospores, and in the presence of parthenospores." Although Randhawa (1943, p. 74) believes that " a difference of a few microns in the width of vegetative cells or in the dimensions of zygospores is quite immaterial in most cases as it may be due to physiological conditions ", even then it is surprising to note that he has created *S. Sahnii*, based upon such differences. It may be pointed out that the shape of the vegetative cells is a variable character, and it is more so when the cells are hypertrophied due to some fungal attack as is found to be the case in the so-called *S. Sahnii* Randh. It is therefore a very risky venture to name a species on a single character, like the presence of parthenospores, especially when it is associated with normal conjugation. Since Randhawa has observed normal conjugation in this alga, the parthenospores appear to be due to the failure of normal conjugation in some filaments. This failure being caused by some internal physiological metabolic upset owing to the attack of a fungus (similar to a species of *Myzocitium*, according to Randhawa). *S. Sahnii* Randh. is, therefore, not a valid species and the characters on which this plant is based are covered by the description of *S. condensata* (Vauch.) Czurda, as the following table shows. In the same paper (*cf.* Randhawa, 1938) where he describes *S. Sahnii* he has also recorded *S. condensata* showing the formation of parthenospores. So, even in the presence of parthenospores, *S. Sahnii* does not differ from *S. condensata*. It is really very surprising how Randhawa describes the same plant differently at two different places in the same paper.

TABLE III

Showing a comparative study of S. Sahnii *Randh. and* S. condensata *(Vauch.) Czurda*

Sl. No.	Characters	*S. Sahnii* Randh. (1938)	*S. condensata* (Vauch.) Czurda (1932)	
			Described by Czurda (1932)	Described by Randhawa (1938)
1	Shape of vegetative cells	"Much swollen and barrel-like" (Appear to be hypertrophied due to fungal attack)	Cylindrical (Unswollen)	Cylindrical
2	Size of vegetative cells (in microns)	48–72×40–74	48–53 in breadth	40–65 in breadth and 2·5 times as long
3	No. of chloroplasts	Single	Single	Single
4	Septa of the cells	Plain	Plain	Plain
5	Fructifying cells	Unswollen	Unswollen	Unswollen
6	Zygospores :—			
	Size (in microns)	22–36×44–68	30×55	32–36×60–70
	Shape	Oval	Langachsig-ellipsoidisch	Oval
	Mesospore	Thick and smooth	Thick and smooth	Brown (Its nature not given but fig. shows it to be smooth)
7	Parthenospores	Present	Unknown	Present

Randhawa mentions about a curious coincidence in this alga. He writes, " It is a curious coincident that both the species (*S. affinis* and *S. Sahnii*) of *Spirogyra*, in which this form of *Myzocitium* has been seen, reproduce by lateral conjugation ". It may be pointed out that this coincidence is a mere chance. There can be, as a matter of fact, no relationship between the mode of conjugation of an alga and its attack by a fungus.

3. *Spirogyra Oudhensis* Randh. (Randhawa, 1938, p. 348).—Randhawa describes the spore-wall in this species having broad reticulations; but his figure 41 *C*, clearly shows that the zygospore is not quite mature. The appearance of the so-called reticulations is due to the changes in the contents of the zygospores during maturation. As the author has already pointed out, the maturation of the spore-wall starts from the periphery of the zygospore and gradually approaches the centre. In the present case the zygospore has reached a stage in which, the mesospore is clearly distinguishable, but the maturation is not perfect. The mesospore in Randhawa's figure, referred to above, is distinctly smooth and the markings are due to immature contents of the zygospores. Again, Randhawa writes that the " mesospore is separated from the other two layers by a considerable space (Fig. C)." This statement is far from being correct when interpreted in the

light of his figure of the zygospore. It appears therefore that Randhawa has failed to understand even a mesospore. *S. Oudhensis*, which is based on the peculiar spore layers and broad reticulations, is therefore invalid and does not deserve to be treated as a new species.

4. *Spirogyra Tandæ* Randh. (Randhawa, 1938, pp. 350–1).—This species appears to be very doubtful since Randhawa's figure (Fig. 44) clearly shows that the zygospore is not mature. The so-called reticulations are due to the immature condition of the zygospore. They are evidently the contents of the spore. It is clearly a species with smooth spore-wall where the mesospore is not yet fully developed. It also appears to be a risky venture to establish a new species on the basis of the dimensions of the cells and the so-called 'brief' conjugation canal.

5. *Spirogyra unduliseptum* Randh. (Randhawa, 1938, pp. 352–3).—This species is based on a single character, *i.e.*, the peculiarly parallelogram-like replicate septum, which is semi-replicate and wavy in nature. The semi-replicate wavy septum is not a constant feature even in the same filament, as is clearly shown by Randhawa's figures (Fig. 46, A and B). It is therefore too much to exploit a single character to establish a new species, such as is liable to a good deal of variation. It is a fundamental mistake to compare this species with *S. Narcissiana* Trans. which reproduces exclusively by aplanospores. The following comparative statement clearly shows that *S. unduliseptum* Randh. is similar to *Spirogyra austriaca* Czurda (1932) in many important characters and does not materially differ from the latter.

TABLE IV

Showing a comparative statement of S. unduliseptum *Randh. and* S. austriaca *Czurda.*

Sl. No.	Characters	*S. unduliseptum Randh.* (1938)	*S. austriaca* Czurda (1932)
1	Size of vegetative cells (in microns)	13–18 broad and 8–12 times as long	14–16 broad
2	No. of chloroplasts	Single	Single
3	Septa	Wavy semi-replicate	Replicate
4	Fruiting cells	Swollen	Swollen
5	Zygospores:—		
	Size (in microns)	20–22×40–50	32×65 ; 26×58
	Shape	Ellipsoidal	Langgestreckt-ellipsoidisch
	Mesospore	Comparatively thicker and yellowish brown, smooth as shown in Fig. 46. In the description Randhawa writes that the spore-wall bears reticulations on its surface, but curiously enough no figure has been drawn to indicate this feature.	Thick, smooth and brown

The former species can at the most be treated as a form of *S. austriaca* which might be named as *S. austriaca* Czurda forma *unduliseptum* form. nov.

6. *Spirogyra Jaoense* Randh. (Randhawa, 1938, pp. 358–9).—A comparison of this species with *Spirogyra Fuellebornei* Schmidle clearly shows that it differs from the same in very minor characters as shown in the following table.

TABLE V.

Showing a comparative statement of S. Jaoense *Randh. and* S. Fuellebornei *Schmidle*

Sl. No.	Characters	*S. Jaoense* Randh.	*S. Fuellebornei* Schmidle
1	Size of Vegetative cells (in micron-)	44–56 in breadth	40–44 in breadth
2	No. of chloroplasts	4–6	3–4
3	Septa	Plain	Plain
4	Fructifying cells	Slightly swollen (Randhawa's figure however does not show any swelling)	Unswollen
	Zygospores :—		40×64–80
5	Size (in microns)	54–58×72–80	
	Shape	Oval to elliptical	Langachsig-ellipsoidisch
	Mesospore	Thicker & brownish	Thick, smooth and brownish
		Exospore, thin & smooth	

S. Jaoense Randh. is therefore a more robust form of *S. Fuellebornei* Schmidle and does not deserve to be a new species.

7. *Spirogyra Manoramæ* Randh. (Randhawa 1938, pp. 360–1).—It is really a very great surprise when one finds that the same plant has been described differently under different names one after the other by the same worker. Even a casual glance at Randhawa's Figs. 55 and 56 will clearly show that *S. Manoramæ* Randh. is exactly similar to *S. submaxima* Trans. var. *inflata* Randh. While discussing the affinities of *S. Manoramæ*, Randhawa writes that " in dimensions of its cells and zygospores this form resembles *S. submaxima* Trans., but it differs from it in having polygonal reticulations on the surface of its zygospores, and its outwardly swollen female cells." Out of the three zygospores of *S. Manoramæ* sketched by Randhawa, the one on the extreme right has been drawn to show polygonal reticulations which is obviously incorrect since the zygospore in question appears to be immature. On the other hand the remaining two zygospores, which have not been described represent the really mature condition, and the spore-wall in these cases is distinctly smooth. With regard to the outwardly swollen nature of the female cells it may be pointed out that similar female cells have been sketched by Randhawa in his variety, *S. submaxima*

Trans. var. *inflata* Randh. (*cf.* Fig. 56, B), in which the inner side is also reported to be inflated, a wrong statement so far as his relevant figure is concerned. It thus appears that the characters which differentiate *S. Manoramæ* from *S. submaxima* are covered up by the description given by Randhawa for var. *inflata* of the latter species, in the light of the correct interpretation of the facts. The only character in which *S. Manoramæ* differs from *S. submaxima* Trans. var. *inflata* Randh. is in the fact that the conjugation canals are lengthened into rhizoidal structures in the latter. The interpretation of this observation appears to be very doubtful and requires verification. On the face of the various evidences *S. Manoramæ* Randh. is not a valid species, and in the author's opinion it should be treated as *S. submaxima* Trans. var. *inflata* Randh. with which it differs in minor respects.

Genus *Zygnema* Agardh

1. *Zygnema Oudhensis* Randh. (Randhawa, 1938, pp. 146–7).—*Z. Oudhensis*, according to Randhawa, differs from *Zygnema Chungii* Li in the presence of both isogamy and anisogamy, and in the size and shape of the spores. The transition between isogamy and anisogamy, *i.e.*, the formation of zygospores either in the conjugation canal or in the female gametangia, is a very variable character and cannot be relied upon. In this connection a reference may be made to a statement made by Fritsch and Rich (1927), while describing the location of the zygospores in *Zygnema peliosporum* Wittr., that " if one and the same species can in a certain year exhibit the one method of conjugation and in another year the other method, the suspicion naturally arises as to whether this feature may be of as little systematic value

TABLE VI

Showing a comparative statement of Zygnema Oudhensis *Randh. and* Zygnema Chungii Li

Sl. No.	Characters	*Z. Oudhensis* Randh. (1938)	*Z. Chungii* Li (1934)
1	Size of vegetative cells (in microns)	22–34×52–70	24–28×68–72
2	Conjugation	Scalariform	Scalariform
3	Zygospores :—		
	Position	Conjugation canal, female gametangia, and also at places between these two, which Randhawa describes as 'unstable location'	Conjugation canal
	Shape	Globose to conical	Globose to ovoid
	Size	34–46 in diameter	38–42×38–47
	Mesospore	With broad reticulations	With irregular reticulate network

in the genus *Zygnema* as the occurrence of scalariform or lateral conjugation is in *Spirogyra* or other members of Zygnemaceæ." It is thus clear that isogamous or anisogamous conjugation in this genus is not an important character for the creation of new species. Even in the matter of size and shape of the spores, *Z. Oudhensis* cannot be very well demarcated from *Z. Chungii* Li, as the above comparative table shows. *Z. Oudhensis* Randh. is therefore not a valid species and can only be treated as a variable form of *Z. Chungii* Li.

2. *Zygnema terrestris* Randh. (*cf.* Randhawa, 1938 and 1940, pp. 147–50).—This plant was collected from moist fields lying fallow on the sides of a freshwater stream. It is very strange that one should find in the same plant aplanospores and zygospores with both reticulations and punctations on the mesospore wall (*cf.* Randhawa's Fig. 29, F, G, and I). This is clearly due to poor observation on the part of Mr. Randhawa. There can be either punctate or reticulate sculpturing, but not both. In the same filaments Randhawa reports aplanospore formation, lateral conjugation and also scalariform conjugation, and still in other cases geniculations. It appears that these are all abnormalities due to forced terrestrial habit of the alga. It is too much to use abnormalities for establishing new species, especially when these abnormalities have not been clearly understood and explained. It may be noted that Randhawa himself, has not been able to differentiate an " aplanospore " from a " laterally produced zygospore." (Fig. 29, E–K). *Zygnema terrestris* Randh. requires revision and is therefore a doubtful species at present.

3. *Zygnema mucigena* Randh. (Randhawa, 1938, pp. 141–2).—While discussing the affinities of this alga Randhawa says that " this species resembles *Z. Carteri* Czurda in the size and shape of vegetative filaments and zygospores; but differs from it in its anisogamous scalariform conjugation." The validity of the importance attached to *anisogamous scalariform conjugation*, the only character on which *Z. mucigena* is based, can be very well judged from the observations already made in connection with *Z. Oudhensis* Randh. *Z. mucigena* Randh. therefore appears to be only a variable form of *Z. Carteri* Czurda and does not deserve to be a new species.

4. *Zygnema giganteum* Randh. (Randhawa, 1938, pp. 144–5).—This species is also not a valid one, the objections being the same as given for *Z. Oudhensis* Randh. nad *Z. mucigena* Randh.

Genus *Mougeotia* Agardh

Mougeotia quadrata Randh. (Randhawa, 1938, pp. 126–7).—While discussing the affinities of the present alga, Randhawa writes that " this form

resembles *M. Regelii* Skuja in the size of cells and zygospores, but differs from it in the shape of its zygospores." Although Randhawa's Fig. 9, showing two zygospores of *M. quadrata* Randh., clearly indicates that these zygospores are not similar in shape, even then a new species has been established on the same character. In his description of the plant he mentions that the " zygospores are quadrate to globose-quadrate ". This is obviously an ambiguous statement, the shape cannot be both globose and quadrate at the same time. *Mougeotia quadrata* Randh., based on a single character, *i.e.*, the shape of the zygospore, which is itself so variable, according to the description and figure given by Randhawa, can hardly be considered a valid species.

Genus *Debarya* Wittrock

Debarya costata Randh. (Randhawa, 1938, pp. 121–2).—In spite of Randhawa having acknowledged that he has not seen mature zygospores in this plant he has described and sketched the zygospore-wall showing reticulations and ribs. The so-called reticulations are due to the immature condition of the zygospore contents, while the mesospore is shown clearly smooth in his figures. The ribs on the other hand are due to foldings of the globose zygospores and should not therefore be given importance in establishing a species. *D. costata* Randh. does not therefore appear to be a new species.

Concluding Remarks

In the beginning of his so-called critical review, referred to previously, Randhawa seems to have advocated certain points with regard to the systematic studies of the group Zygnemales. According to him the sculpturing of the spore-wall is of fundamental importance particularly in species of *Zygnema, Spirogyra* and *Sirogonium*. It is not only in these genera but also in other Zygnemales that the mesospore-sculpturing is of vital importance as a systematic character. Although the sculpturing of the mesospore is an important character it is rather inadvisable to exploit too much a single character unless it is of a singular nature as one can easily understand and infer from Czurda's Zygnemales in "Pascher's Süsswasser-flora, Mitteleuropas, Heft 9, 1932 ", the only standard work on the systematic study of this group. It may be pointed out in this connection that, in spite of Randhawa's helpful tips (*cf.* Randhawa, 1943, p. 74), which he has, in fact, borrowed from Jao (*cf.* Jao, 1935, p. 561), it is sometimes difficult to identify the nature of the mesospore, especially when it is not well-defined due to a number of types of sculpturing occurring together in the same spore. Under such circumstances, however, if one follows the process of maturation of the zygospore clearly it is quite possible to interpret and recognise the mesospore sculptur-

ing correctly. The spore-wall starts maturation from the periphery and it gradually proceeds towards the centre of the spore. The first layer to be differentiated is the exospore which in most cases is thin, hyaline and smooth and sometimes double-layered. The next to appear is the mesospore which is thick, often variously coloured, although sometimes colourless at maturity. It may be in several cases two to three layers in thickness and with varied ornamentations, but in many cases it has been found to be smooth in the genus *Spirogyra*. If it is ornamented it is possible to make out the nature of the markings even if the mesospore is not fully formed as it begins differentiation from the place where the exospore is completed. The failure of the recognition of this point and the examination of the zygospore-wall in the middle of the spore sometimes leads to erroneous interpretations about the nature of sculpturing especially when the zygospore contents are immature. In such cases it has been often observed that the zygospore-wall appears to possess broad reticulations but it is not a fact. It is on this account that Randhawa has, in many cases, in the genera *Spirogyra*, *Zygnema* and *Debarya*, described the mesospore with broad reticulations, although he realizes that the contents look opaque when fully mature. The last layer to be differentiated is the endospore which is generally thin and indistinct.

While referring to the number of chloroplasts in *Sirogonium inflata* Dixit and *S. indicum* Singh, Randhawa writes that " to pick up any individual cell and to say that it contains 7 or 10 chloroplasts and then to base a new species on it is rather a risky venture." It should be noted that it is never so that any individual cell is picked up and the number of chloroplasts is reported in terms of the same. As a matter of fact a number of cells, from many lots of the material in question, are examined then a definite statement is given. If there is a variation in the number of chloroplasts the range of variation is clearly mentioned. In the cases, referred to above, the number of chloroplasts has been found to be fixed, either ten or seven. Again, it is not only on a single character, *i.e.*, the number of chloroplasts or any other feature, that a new species is based but on a combination of characters, and that too when the characters have been carefully and repeatedly studied from both morphological and ecological points of view.

The failure of the recognition of ecological conditions under which an alga is growing leads, in many cases, to erroneous interpretation. It appears that the terrestrial habit of an alga is sometimes ill-defined. In many cases it has been found that the plants actually growing in water in the beginning are left over on the mud or soil when the water has receded or dried up, and under such circumstances the algæ have been described to possess terres-

trial habit (*cf.* Randhawa,1938, 1940 and 1941). It is wrong to describe such forms as terrestrial unless their growth-cycle is studied and found to be so from the beginning. In fact they are forced to possess a certain habit, and under these conditions they should be considered to be only lingering rather than growing. This forced habit in the majority of cases leads to abnormalities in vegetative structures as well as in reproductive parts, and these abnormalities, which should not be exploited, have been made use of by Randhawa in creating new species, in addition to the abnormalities occurring in nature even under normal conditions. There is no doubt that there are terrestrial forms which grow on sprayed rocks or on moist soil over which a thin layer of water is gently trickling.

In conclusion, it may be said that Randhawa's findings should be treated with considerable reserve. For the conduct of research on the Indian Zygnemales, reference should be made to the standard works of Borge and Pascher (1913), Czurda (1932, 1937), and Fritsch and his collaborators (1918, 1921, 1922, 1924, 1927, 1930 and 1935), together with papers by Transeau and his co-workers, (1925, 1932, 1933, 1934 and 1938; 1934), Iyengar (1923, 1932), and Jao (1935 1936).

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1402.46—Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore,